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DENITRIFICATION IN TURF

A Dissertation Presented

By

CHARLES FRANCIS MANCINO

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

February 1986

Plant and Soil Sciences

Charles Francis Mancino

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Massachusetts Experiment Station

Project Number 526

DENITRIFICATION IN TURF

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CHARLES FRANCIS MANCINO

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DEDICATION

To Lori, whose support and understanding made it all possible. 143.

ACKNOWLEDGEMENTS

I would like to thank the faculty and staff of the Department of Plant and Soil Sciences for their assistance in completing this research project. During my stay at the University I have had to call upon all of them for advice, equipment, supplies and friendship.

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This research has required equipment which did not personally belong to the Turf Research Laboratory. The Varian GC, used to determine N_2O-N concentrations, was made available by David Houle and the rest of the staff at West Experiment Station. This project would not have been possible without their assistance and instruction. Dr. Lyle Craker allowed us the use of his Shimadzu GC for C_2H_2 determination and the use of much computer soft/hardware. Dr. David Wehner, from the University of Illinois, supplied six of the acrylic turf chambers. Thank you all very much.

Research equipment is not the only requirement for success in graduate school. The handling of funding, ordering of supplies and equipment and the use of departmental office equipment is also a

necessity. This facet of research was graciously provided by Mrs. Marion Lemay and Mrs. Deborah Clark.

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ABSTRACT

Denitrification in Turf

February, 1986

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The loss of nitrogen(N) from agricultural soils due to the process of denitrification may be extensive after fertilizer-N applications. To date, no research has been conducted to directly assess denitrified-N losses from turfgrass areas.

Denitrifying soil microbial populations in unsaturated and saturated silt and silt loam soils underlying Kentucky bluegrass (Poa pratensis L. var. 'Baron') were enumerated using the most-probable-number technique. Populations ranged from 22.6×10^5 to 99.9×10^5 g/dry soil and 7.4×10^3 to 61.0×10^3 g/dry soil in the unsaturated silt and silt loam soils, respectively. Soil saturation resulted in an 87-fold increase in the denitrifier population in the silt soil and a 121-fold increase in the silt loam soil. Soil depth (0-15 cm) and nitrate addition (48.8 kg N/ha) did not significantly influence denitrifier number.

Denitrification losses were directly measured from sod samples sealed in acrylic chambers. The acetylene inhibition technique was utilized and each study lasted ten days. Losses from both soils

at 22°C were less than or equal to 0.10% of the applied potassium nitrate fertilizer (48.8 kg N/ha) when soil moisture levels were 75% of soil saturation or less. Saturated soil conditions increased denitrification losses from the silt and silt loam soils to 5% and 2% of the applied N, respectively.

A linear relationship existed between denitrification losses (0.02, 0.06 and0.10% of applied N) and soil temperatures between 22° and 30° C in the silt soil at 75% of soil saturation. Soil temperatures of greater than or equal to 30°C and saturated soil conditions resulted in losses equivalent to 85% and 41% of the applied N from the silt and silt loam soils, respectively. Denitrification losses did not increase at soil temperatures above 30°C.

Soil saturation following the nitrification of urea (48.8 kg N/ha) for five days resulted in denitrification losses of 5.5% of the applied N from sod on the silt soil incubated at 22°C.

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INTRODUCTION

Denitrification occurs in soil, water and sediment. A prequisite for denitrification is the establishment of anaerobic conditions. Under these conditions some facultative, anaerobic microorganisms have the capacity to utilize nitrate (NO_3^-) or nitrite (NO_2^-) as terminal electron acceptors in respiration. Nitrous oxide (N_2O) and dinitrogen (N_2) are the gaseous end products of this stepwise reduction. At present the mechanism of this process appears to be:

$$2\ HNO_3^- \xrightarrow{+4H^+} 2\ HNO_2 \xrightarrow{+2H^+} 2\ NO \xrightarrow{+2H^+}$$

$$N_2O \xrightarrow{+2H^+} N_2 \text{ (26,28,31,38,61).}$$

Nitrous oxide and dinitrogen may eventually be lost to the atmosphere.

The gaseous loss of nitrogen from soil, water and sediment through the denitrification process is an integral part of the nitrogen cycle. It also represents the loss of an important plant nutrient from agricultural soils. The chemical fixation of atmospheric nitrogen in plant fertilizers requires the use of fossil fuel energy sources and, therefore, is costly. As such, it is

important to understand the fate of nitrogen fertilizers applied to agricultural soils and how various cultural practices affect nitrogen losses.

Denitrification losses after nitrogen fertilizer applications to field and vegetable crops (3,18,48,62), grasslands (17,25,42,43,46,47,67), fallow soils (3,27,42) and flooded soils (24,27,52) have been studied extensively. Small, frequent irrigations (43,50,51), nitrogen fertilizer applications (4,17,18,46), high soil organic matter levels (7,12,57) and plant root respiration (58,62) have been shown to stimulate denitrification. These conditions are often found during the production and maintenance of high quality turf. No research has been reported on denitrification potentials in lawn and recreational turf even though available estimates indicate that the turf industry is a large component of the American economy (6). Until information regarding the effects of environmental and cultural practices on denitrification in turf is obtained, accurate nitrogen fate studies and efficient nitrogen fertilizer recommendations will be incomplete.

The objectives of this research were: a) to enumerate the denitrifying population of a saturated and unsaturated silt and silt loam soil under fertilized and unfertilized Kentucky bluegrass turf; b) to determine the

influence of acetylene, which is utilized in denitrification studies, on denitrification in small turf soil samples and large sod samples and c) to directly measure denitrification loss from Kentucky bluegrass sod as influenced by soil texture, soil moisture content, soil temperature and nitrogen fertilizer type.

C H A P T E R II

ENUMERATION OF DENITRIFYING MICROBIAL POPULATIONS IN TURF

Introduction

Gaseous loss of soil nitrogen through the process of denitrification is mediated by a wide variety of heterotrophic, facultative, anaerobic microorganisms including the genera Pseudomonas, Achromobacter, Micrococcus and Bacillus (13,38). The presence of large populations of denitrifiers in well aerated soils may not be indicative of concurrent denitrification activity since these organisms are facultative anaerobes reducing nitrate and nitrite only under anaerobic soil conditions. Large denitrifying microbial populations may, however, represent a greater potential for gaseous N-loss if soil conditions become partially anaerobic during frequent or extended periods of rainfall or irrigation (3,30). Such conditions are typical of highly managed turf areas which require frequent irrigation as well as nitrogen fertilization (6).

Both rainfall and irrigation events are known to induce temporary anaerobic soil conditions (43,48). Frequent irrigation events as well as repeated periods of

soil wetting and drying have been shown to significantly increase the extent of denitrification (30,54).

Several factors aside from increased soil moisture levels are known to influence the extent of denitrification in soils. Soils having high organic matter contents enhance N-loss through denitrification by providing increased levels of organic carbon as energy substrate for heterotrophic microbial respiration (3,28,38,43). Increased rates of microbial respiration further reduce soil oxygen levels (15,33). In addition, extensive root systems that are actively growing have been shown to enhance the rate of denitrification due to an increase in oxygen demand (58,62). Intensively managed turfgrass areas typically possess extensive and prolific root systems which normally contribute high levels of organic matter to the soil through root exudation and decay associated with yearly turfgrass turnover (6). Therefore, the nature of a turf rhizosphere combined with the regular use of nitrogen fertilizers and irrigation most likely provide an ideal environment for supporting large denitrifier populations.

Standard methods, including the most probable number (MPN) estimate, have been used successfully in enumerating soil denitrifier populations (1,22,64). Measurement of the disappearance of nitrate (NO_3^-) from a NO_3^- enriched

nutrient broth inoculated with serial dilutions of soil extract is the most common procedure. Nitrate removal, however, may or may not be an indication of the presence of denitrifiers because soil NO_3^- respirers can also reduce NO_3^-. Strict NO_3^- respirers can not reduce this nitrogen substrate beyond nitrite (NO_2^-). Therefore, NO_3^- enriched nutrient broths may cause a false positive result for the number of denitrifiers unless the broth is also tested for NO_2^-. Nitrite enriched nutrient broths overcome this problem and have been used successfully in the enumeration of denitrifiers (61). Denitrifying microbial populations have been enumerated for various cropped and fallow soils and, in general, populations range between 1.3×10^3 and 8.9×10^5 per g dry soil (3,10,22,56,61).

Denitrifier populations for sand:peat:sandy loam mixtures utilized on golf course putting greens never exceeded 4.9×10^3/g dry soil (36). Such low populations were undoubtedly due to the low amount of soil associated with these well drained soil mixtures. It seems likely that natural turf soils would contain higher denitrifier populations than sand:peat mixtures.

The objectives of this study were to enumerate the denitrifying microbial population of a saturated and unsaturated silt and silt loam soil under N-fertilized and unfertilized Kentucky bluegrass.

Materials and Methods

A 5-year old stand of a Kentucky bluegrass (*Poa pratensis* L. var. 'Baron') turf grown on either a Hadley silt or Hadley silt loam soil (typic, mesic, udifluvent) was used in this study. Physical properties of both soil types are listed in Table 1. Experimental plots measured 1.5 x 1.5 m and were arranged in a completely randomized design including 3 replications per treatment. Fertilizer (potassium nitrate) and irrigation treatments were a) 48.8 kg N/ha only; b) 48.8 kg N/ha with daily irrigation of 7.6 cm water; c) daily irrigation of 7.6 cm water only; and d) control plots without fertilizer or irrigation. The relatively high level of irrigation treatment was used to insure temporary soil saturation on a daily basis during the 8 day experimental period.

Aluminum cylinders measuring 35.6 x 30.5 cm (H x I.D.) were inserted to a depth of 20.5 cm into the center of each plot designated for irrigation treatments. Application of water to turf inside these cylinders allowed for greater application accuracy as well as limiting the extent of lateral water movement. Tensiometers were installed at soil depths of 3 and 10 cm within each cylinder. Soil saturation (0.0 MPa) was, in general, attained within 2 hours after irrigation for both

Table 1. Characteristics of Hadley silt and silt loam soils.

	Soil Type	
	Silt	Silt Loam
Particle Size, %		
Clay	6	4
Silt	83	79
Sand	11	17
Organic Matter, %	5.0	3.9
Infiltration Rate (cm/h)	10.9	6.9
% of Soil Saturation at Field Capacity	56.8	51.8
pH	6.35	5.79

soil types.

Nitrogen fertilizer applied to plots not designated for irrigation was washed into the turf with 1.3 cm of water at the start of this experiment.

Five soil cores (2.5 x 30 cm, D x L) were taken from within each plot for analysis 8 days after treatment initiation. Cores were divided into 3 soil depths (0-5 cm, 5-10 cm and 10-15 cm) measured from below the existing turf thatch layer and mixed to form a single composite soil sample for each depth. Soil moisture content was determined gravimetrically on composite subsamples.

Subsamples (10 g moist soil) from each composite soil sample were placed into 90 ml of sterile, distilled water and shaken for 5 minutes. After dispersion, a 10-fold dilution series (v/v) ranging from 10^{-2} to 10^{-7} was made. Two ml of each soil suspension were injected using a sterile syringe into test tubes sealed with serum vial caps. Each tube contained 16 ml of sterile nitrite nutient broth (8 g Difco nutrient broth containing 10 mM nitrite). The headspace of each tube was evacuated of air and replaced with dinitrogen gas immediately prior to inoculation. Five tubes were inoculated per dilution and incubated in the dark at 30 ^{o}C for 7 days.

Following incubation, the inoculated nutrient broth was tested for the presence of nitrite using NEDD

(N-1-napthyl-ethylenediamine)- sulfanilamide reagent (63). The presence of nitrite signified the absence of a denitrifying population. The most probable number (MPN) method (1,64) was used to estimate the denitrifier population/g oven-dry soil.

Random spot tests of inoculated broth were made for the presence of nitrate using a reducing agent followed by the test for nitrite (63). An indophenol method was used to determine the presence of ammonium (14). These random tests were performed to insure that nitrite transformation to either nitrate or ammonium was not occurring under the imposed anaerobic incubation period.

Results and Discussion

Soil type as well as water treatment significantly influenced denitrifier populations with the silt soil supporting higher populations than the silt loam soil (Table 2). Maximum populations were 99.0×10^5 and 944×10^5 denitrifiers per g soil in the silt soil compared to 0.61×10^5 and 247×10^5 denitrifiers per g soil in the silt loam for saturated and unsaturated soil conditions, respectively (Table 3). These differences were most likely the result of the higher soil moisture content of the silt soil under non-watered conditions. Following eight days

Table 2. Analysis of variance for the effects of soil type, depth, irrigation and nitrate fertilization on denitrifier populations.

Source	df	Significance of F Value+	
		Silt	Silt Loam
Soil	1	**	
Treatment	3	**	**
Nitrate	1	NS	NS
Irrigation	1	**	**
Nitrate with Irrigation	1	NS	NS
Depth	2	*	NS
Treatment x Depth	6	NS	NS

+ F values determined with orthogonal single degree of freedom comparisons.

*,** Significantly different at the 5% and 1% probability levels, respectively.

NS Non-significant at the 5% probability level.

Table 3. Variation in denitrifying microbial populations in turf.

Treatment	Soil Depth, cm	Denitrifier Number, x10^5/g dry soil*	
		Silt Loam Soil	Silt Soil
Unsaturated Soil			
(+) Nitrate	0-5	0.17 ± 0.09	24.7 ± 5.4
	5-10	0.09 ± 0.04	26.8 ± 5.1
	10-15	0.33 ± 0.19	51.9 ± 18.0
(-) Nitrate	0-5	0.07 ± 0.03	22.6 ± 2.9
	5-10	0.61 ± 2.1	25.5 ± 9.8
	10-15	0.19 ± 1.4	99.0 ± 16.0
Saturated Soil			
(+) Nitrate	0-5	19.6 ± 5.4	335 ± 13
	5-10	11.9 ± 5.0	652 ± 255
	10-15	22.5 ± 2.0	461 ± 109
(-) Nitrate	0-5	83.0 ± 44.3	662 ± 260
	5-10	16.8 ± 6.9	944 ± 246
	10-15	247.0 ± 11.5	416 ± 27

*Data represents the mean of 3 replications ± standard error.

without watering, the soil moisture content of the silt loam soil decreased to 17.5% of saturated soil conditions while the silt soil remained high at 44.9% of saturation. The relatively high soil moisture content associated with the silt soil may have increased the number of anaerobic microsites within this well-aggregated soil. Therefore, the silt soil may be more favorable for supporting anaerobic denitrifier populations than the silt loam, particularly during periods of low water input. Also the higher soil organic matter content and pH of the silt soil cannot be ruled out in contributing to these soil microbial populations (Table 1).

Saturated soil conditions have been shown to enhance the denitrification process (27,54). Anaerobic soil conditions, even if only within aggregate soil microsites, can enhance denitrification (61). Our results show that the saturation of a silt loam soil and silt soil greatly increase the number of denitrifiers (Table 3) and, thus, may increase the potential for gaseous N loss through denitrification.

Additions of nitrate under anaerobic soil conditions can influence the type and extent of gaseous N effluxes (15,52), however, it is not clear as to how added nitrate influences the number and diversity of denitrifiers (26). The addition of fertilizer nitrate did not significantly

increase denitrifier populations in both soils tested (Table 2). In a previous study addition of nitrate fertilizer increased soil denitrifier populations but these results were complicated by additions of various carbon substrates (64).

Denitrifier activity between different soil depths usually reflects the distribution of soil organic matter, soil moisture content and level of soil aeration (28). Although the distribution of denitrifiers between soil depths was not statistically different (Table 2), larger populations were noted at the lower 10 to 15 cm soil depths for three of the four unsaturated treatments (Table 3). Irrigation to the point of saturation resulted in somewhat higher populations within the upper 5 to 10 cm depths of the silt soil (Table 3). These results are most likely due to the increased level of soil moisture at these depths as related to water treatment.

Denitrifying microbial populations within turf soils tested in this study were, in general, higher than those found for other agronomic soils (3,22,52,61). Denitrifier populations in a sandy loam putting green soil mixture (70% sand, 20% peat, 10% sandy loam soil) were found to be significantly lower than populations reported in this study (36). The relatively low populations of this previous study were, however, most likely due to low soil

content of this well drained soil mixture.

Intensively managed turf areas normally retain high levels of soil organic matter through seasonal decomposition of older roots, shoots, rhizomes or stolons (6). Therefore, the relatively high level of organic matter maintained within a turf root zone can provide adequate carbon substrate necessary for supporting a large and active microbial population. A high rate of microbial activity combined with the rapid growth of an extensive, fibrous turfgrass root system most likely enhances the establishment of anaerobic soil conditions. Active root growth coupled with increased rates of microbial respiration are known to significantly reduce soil oxygen levels (15,33,58). Frequent or prolonged periods of rainfall or irrigation would further decrease the level of soil aeration. Results of this study have shown that saturated soil conditions, especially in finer textured soils, can support large populations of denitrifying microorganisms. Frequent and heavy irrigation events coupled with nitrogen fertilization may, therefore, increase the potential for nitrogen loss by denitrification.

C H A P T E R III

EFFECTS OF ACETYLENE ON A TURF SOIL

Introduction

The predominant product of denitrification is dinitrogen (N_2). Because N_2 comprises 78% of the Earth's atmosphere it is difficult to measure small fluxes in N_2 concentrations above soil unless ^{13}N or ^{15}N are utilized.

Nitrous oxide (N_2O) is the precursor of N_2 during the denitrification process (29). Nitrous oxide reductase is responsible for the reduction of N_2O to N_2 and is non-competitively inhibited by acetylene (C_2H_2) (5,35). Concentrations of C_2H_2 as low as 0.1% (v/v) are known to be effective in inhibiting N_2 formation (8,49,53). Because ambient levels of N_2O are normally very low (3 to 5 ppm) small changes in N_2O concentrations above the soil surface can be measured using sensitive gas chromatographic techniques (30).

Although the C_2H_2 blockage technique has been an important tool for denitrification research, its use has been criticized because: a) C_2H_2 blocks nitrification, b) C_2H_2 may be metabolized by soil microbes and c) C_2H_2 blockage of nitrous oxide reductase may have short term effectiveness.

Acetylene has been shown to be as effective as N-Serve [2-chloro-6-(trichloromethyl)pyridine] in its blockage of nitrification (4) with ammonium oxidase as the most sensitive enzyme (60). Acetylene, therefore, may indirectly influence the denitrification process by preventing the formation of NO_3^--N substrate. Nitrification blockage would also lower the O_2 demand in the soil created by nitrifiers (65). However, the blockage of nitrification by C_2H_2 in denitrification field studies has not been shown to occur. Ryden and Lund (48) found very close agreement between direct and indirect estimates of denitrification losses utilizing C_2H_2 blockage. In another study Ryden (47) found the nitrogen distribution in a grassland soil not to be affected by C_2H_2 usage. These results may indicate that the C_2H_2 blockage technique's influence on nitrification may not pose a major problem in field studies or laboratory studies involving large soil samples.

Increases in readily available soil organic matter stimulate the denitrification process (28,43). Acetylene may also serve as a carbon source for many soil and estuarine sediment microorganisms (21,23). The end products of this little understood process may be fermentative, CO_2 or incorporation into soil organic matter (60,66,69). Ethylene is most likely not a

constituent of this metabolic process (66). The enhancement of soil microbial respiration by the presence of C_2H_2 could stimulate the denitrification process by increasing soil O_2 demand (69). Such a problem with the C_2H_2 blockage technique has not been shown conclusively. Smith et al. (53) found similar results for denitrification between the C_2H_2 blockage technique and ^{13}N method in which CO_2 levels were not different in flasks regardless of C_2H_2 presence. Ryden et al. (49) found similar results. These results contrast with those of Yeomans and Beauchamp (69) who found denitrification losses increased as a result of C_2H_2 addition. Carbon dioxide production did not account for lost acetylene.

Despite the lack of evidence suggesting that C_2H_2 may serve as a substrate in denitrification, its disappearance in soil has been observed under both laboratory and field conditions. This consumption occurs very rapidly in anaerobic soils and, on average, after a 7-day lag phase in aerobic soils (21,60,66,69). Aerobic soils made anaerobic did not exhibit acetylene loss (60).

The addition of organic matter to soil may reduce C_2H_2 decomposition. Amendments of alfalfa (1% w/w) to soil prevented C_2H_2 disappearance (69). Terry and Duxbury (60) also found that the addition of alfalfa retarded C_2H_2 utilization in C_2H_2 adapted soils, but enhanced C_2H_2

consumption 4-fold in unadapted soils. This information suggests that some type of co-metabolism may be required between C_2H_2 and other types of carbohydrates for C_2H_2 decomposition in C_2H_2 unadapted soil while other types of carbohydrates may substitute for C_2H_2 in adapted soils thereby reducing acetylene decomposition.

Nitrogen source and amount can influence C_2H_2 consumption in soils. Ammonium, NO_2^- and NO_3^- salts stimulate this process at lower concentrations (10 mg N/ kg soil) (60). These results are most likely due to stimulation of soil microbes, especially those capable of metabolizing C_2H_2. Concentrations of ammonium (NH_4^+) up to 1000 mg N/kg soil stimulated C_2H_2 utilization (60,66) but concentrations of NO_2^- and NO_3^--N greater than 10 mg N/kg soil greatly reduced C_2H_2 disappearance (60). This suggests that denitrification studies involving added NO_3^--N would reduce the possibility of interference from acetylene metabolism.

The effectiveness of the C_2H_2 blockage technique over prolonged periods of time has been questioned. It has been suggested that denitrifiers may switch their metabolic process to overcome inhibition of nitrous oxide reductase (39,59,68). Yeomans and Beauchamp (68) reported that after 168 hours of exposure to C_2H_2 (0.1 and 1.0% v/v) microorganisms were able to reduce N_2O to N_2. The

reduction of N_2O in the presence of C_2H_2 suggests that there is some metabolic change or the growth of microbial species which may cause the reduction of N_2O in the presence of C_2H_2. If this is the case, measurement of N_2O emissions using the C_2H_2 blockage technique would be limited to short time periods (i.e. less than 7 days). Some researchers have not found acetylene acclimatization of soil microbes to occur (47).

Because the influence of C_2H_2 on soil microbial processes is variable, it is necessary to study the effects of C_2H_2 on a turf soil with physical, chemical and microbial properties that suggest a large denitrification potential. The objectives of this research were to show: a) the effectiveness of the C_2H_2 blockage technique in large sod samples contained in experimental chambers, b) the ability of C_2H_2 to block the nitrification process in small soil samples as well as large sod samples and c) the effects of soil moisture content and NH_4^+-N and NO_3^--N ammendments on acetylene disappearance in a turf soil. The acclimatization of denitrifiers to acetylene is discussed in Chapter 4.

Materials and Methods

All studies were conducted on a Hadley silt soil (Table 1) which was situated under an established stand of Kentucky bluegrass (*Poa pratensis* L. var. 'Baron'). The denitrifying population of this soil ranged from 22.6 x 10^5 to 99.0 x 10^5/g dry soil under unsaturated soil conditions and from 335 x 10^5 to 944 x 10^5/g dry soil when saturated.

Measuring the Effectiveness of the Acetylene Blockage Technique

A comparison was made between the N_2O-N emissions of C_2H_2 treated or untreated sod sealed in acrylic chambers. Detailed descriptions of chamber design, sod installation, C_2H_2 introduction into sod and gas sampling procedure and analysis are found in Appendices A to H.

Sod samples were 30.5 x 30.5 x 7.6 cm (L x W x D) with a fresh weight of 7.7 kg. Initial soil moisture content was 66.1% of saturated soil conditions (48.4% water, wt/wt). Potassium nitrate was applied at a rate of 3.02 g/sod sample (4,886 mg N/m^2) in 600 ml of water. Final soil moisture content was 78.5% of saturation.

Following N fertilizer application the chamber lids were sealed and headspace gas was collected and analyzed

for N_2O-N content at 12 hour intervals. Soil temperature was maintained at 22 ^{o}C (day/night). There were 3 replications/treatment.

Inhibition of Nitrification by Acetylene and the Disappearance of Acetylene in Soil in the Presence of Ammonium-N and Nitrate-N

Experiment I. Six gram samples of sieved (<2 mm) air-dried silt soil were placed into test tubes (23 ml volume) and brought to 50, 70 or 90% of saturation by the addition of 1.5, 2.0 or 2.6 ml of distilled, deionized water. Designated samples received an $(NH_4)_2HPO_4$ solution (100 ug NH_4^+-N/g dry soil) instead of water. All samples were slowly wetted by dispensing liquids into the soil centers using Pasteur pipettes. The tubes were then sealed with rubber septa and half of them were inoculated with 0.23 ml of atomic absorption grade C_2H_2 (99% pure). Initial acetylene levels were bewteen 0.8% and 0.9% (v/v) of the soil atmosphere. After a 24 hour period of equilibration at 4 ^{o}C the concentration of C_2H_2 in 0.2 ml headspace samples was determined using a gas chromatograph equipped with a flame ionization detector (Appendix E). All samples were then incubated in the dark for 10 days at 25 ^{o}C.

Headspace levels of C_2H_2 were measured 2, 4, 6, 8

and 10 days after incubation was begun. At the end of the incubation period soil NO_3^--N levels were measured using an Orion NO_3^- specific electrode (Model 93-07, Orion Research Inc., Cambridge, MA, USA). Nitrate was measured in a 1:5 soil:water solution devoid of ionic strength adjuster (ISA), silver sulfate (for chloride interference) and boric acid preservative solution. Following NO_3^--N determinations NH_4^+-N was extracted from the soil by agitation of the samples for 2 days in 200 ml of a 10% NaCl solution. The extract was filtered and NH_4^+-N was measured using an indophenol reaction (14).

Each treatment had 5 replications. Sterile controls were also incubated to test for tube leakage of C_2H_2 as well as background levels of soil NH_4^+-N and NO_3^--N. Chow's Procedure (16) was utilized to make comparisons between the C_2H_2 disappearance rate of NH_4^+-N treated and untreated soil at each moisture level. An analysis of variance (ANOVA) was used to compare the final NO_3^--N levels of acetylene treated and untreated samples.

Experiment II. Six gram soil samples were amended with either 0, 50 or 100 ug KNO_3-N/g dry soil. Nitrogen treatments were applied in 2.0 ml of water for a final soil moisture content of 70% of saturation. Tubes containing soil were sealed, inoculated with C_2H_2 and incubated as described in the previous study.

Acetylene concentrations in tube headspaces were measured and statistically compared to C_2H_2 levels in similarly treated sterile controls using ANOVA. Linear regression analysis was conducted across treatments. There were 5 replications/ treatment.

Experiment III. A test for the disappearance of C_2H_2 in a saturated, anaerobic soil was made. Six gram soil samples contained in test tubes were saturated with 6 ml of water. Tubes were sealed and flushed three times with N_2 gas and then inoculated with C_2H_2. After a ten day incubation period a statistical comparison was made between the C_2H_2 concentrations of tubes containing sterile (120 ^{o}C, 1 kg pressure/cm^2, 15 minutes) and unsterile soil using ANOVA. There were 5 replications/treatment.

Experiment IV. Nine sod samples were installed into the acrylic chambers in order to test for the inhibition of nitrification by C_2H_2. Each of six samples received 0.97 g of urea-N (4,886 mg N/m^2) delivered in 600 ml of water. Two other samples received water only and the remaining sod sample received 3.02 g of KNO_3. Final soil moisture content of the sod samples was 52.3% of soil saturation. All but three urea-treated samples were exposed to acetylene.

Three soil samples were collected from each chamber

1, 3, 5, 7 and 9 days after N-fertilizer application using a cork borer (1.2 cm diameter). Soil samples were combined from each box to give one composite sample/day/replicate. The soil was oven-dried (30 °C, 24 hours) and NO_3^--N determinations were made using 1:5 soil:water solutions.

Results and Discussion

Effectiveness of Acetylene Blockage Technique

Acetylene was found to be an effective inhibitor of N_2O reductase despite the large sod sample size. Nitrous oxide flux was much greater in C_2H_2 treated sod than the untreated sod (Figure 1). Total N_2O-N loss was significantly greater (4.9 fold) in C_2H_2 treated sod (Table 4). Concentrations of N_2O were also detected for an additional day in acetylene treated sod.

Small quantities of N_2O-N were produced even in the absence of C_2H_2. These results indicate that under certain soil conditions some N_2O-N would normally be lost from the soil, but it is apparent that a majority of the denitrification losses would have been in the form of N_2 as represented by differences between the two curves in Figure 1. Dinitrogen losses have been found to be as high as ten times those of nitrous oxide-N (19).

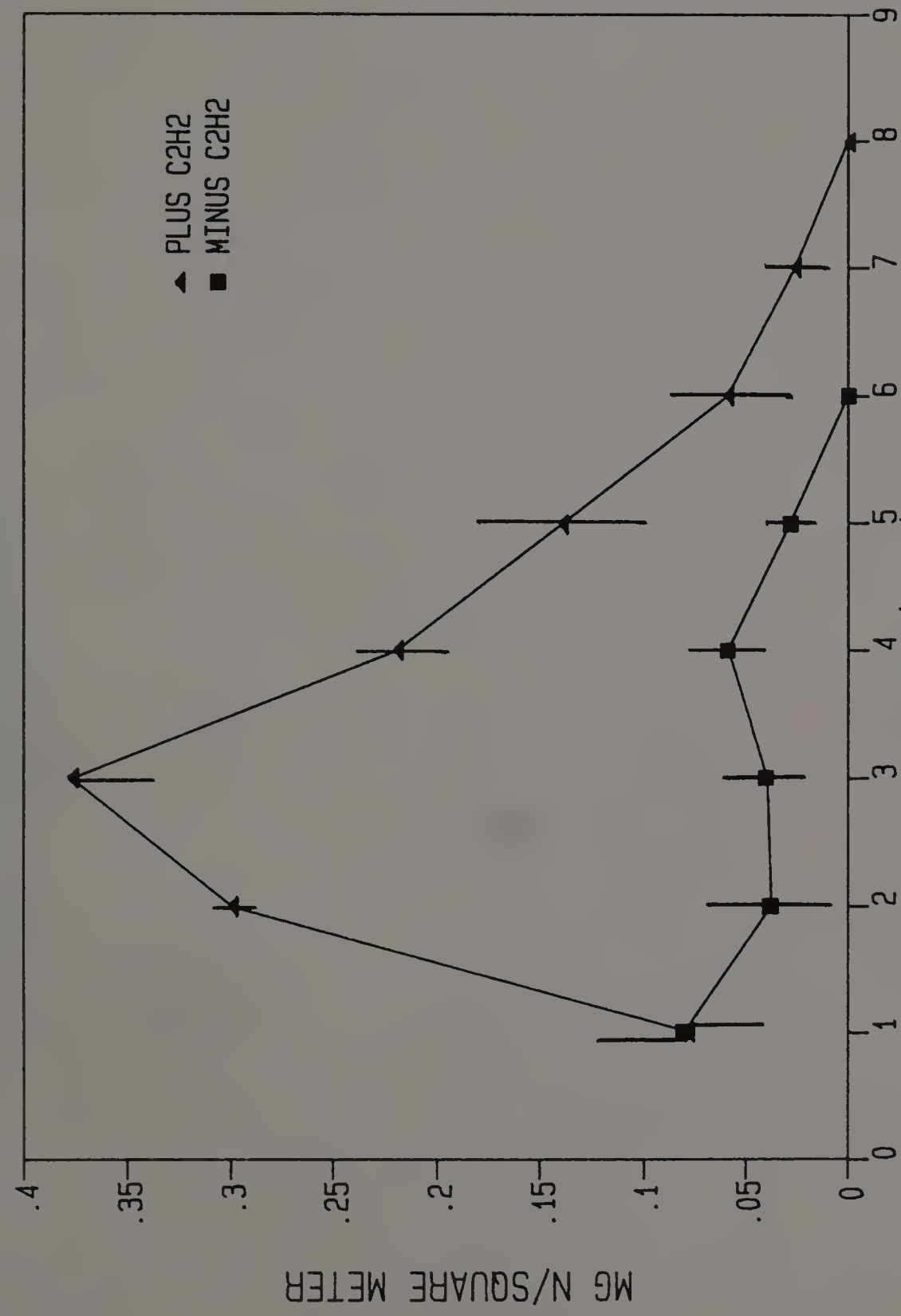

Figure 1. N_2O-N efflux from Kentucky bluegrass sod on a silt soil (75% saturated; 22°C) in the presence or absence of C_2H_2 (1% v/v) following KNO_3 – N addition (4886 mg N/m^2). Vertical lines represent standard error.

Table 4. Total N_2O-N losses from KNO_3 treated (48.76 kg N/ha) sod on a silt soil (75% saturated) with (+) or without (-) C_2H_2 (1% v/v)

C_2H_2	mg N_2O - N/m^2	
+	1.20 ± .22†	*
-	0.25 ± .12	

† Mean of 3 replicates ± SE.
* Significantly different at the 5% probability level.

Inhibition of Nitrification by Acetylene

Nitrification was completely inhibited in soil by C_2H_2 at soil moisture contents of 50% and 70% of saturation but not in soil which was unexposed to C_2H_2 (Table 5). When soil moisture content was 90% of saturation the nitrification process was completely inhibited both with and without C_2H_2 as a result of the inhibiting effect of an O_2 deficiency. Mineralization was not inhibited by C_2H_2 and NH_4^+-N was found to accumulate in tubes in which nitrification was prevented. Similar results were found by Ryden (45).

In accordance with this study, C_2H_2 has been shown by others to be an effective inhibitor of nitrification (37,47,65). Concentrations as low as 0.01% C_2H_2 (v/v) were inhibitory (8). Levels of C_2H_2 used in denitrification studies generally range from 0.1% to 10% (v/v) with higher concentrations being used in soils containing less than 3 ppm of NO_3^--N (53). However, under field conditions the inhibitory influence of C_2H_2 on nitrification may not occur (47).

Because the sod samples used in these denitrification studies were large (7.7 kg), it was necessary to evaluate the effects of continuous exposure of C_2H_2 on nitrification in these samples. Urea was not nitrified in C_2H_2 exposed sod and soil NO_3^--N levels were

Table 5. Nitrate produced and ammonium remaining in Hadley silt soil at 3 soil moisture levels (% saturation) after 10 day incubation period with (+) or without (-) 100 µg NH_4^+-N and 1% (v/v) C_2H_2.

	% Soil Saturation					
	50%		70%		90%	
	NH_4^+ - N	NO_3^- - N	NH_4^+ - N	NO_3^- - N	NH_4^+ - N	NO_3^- - N
Sterile Control	19.59 ± 1.23†,††	4.29 ± .90	22.58 ± 7.48	4.69 ± .98	23.75 ± 5.46	4.50 ± .61
Unsterile Soil						
(+)NH_4^+ (+)C_2H_2	141.37 ± 2.87	4.99 ± .97	134.41 ± 4.71	4.84 ± .23	128.18 ± 3.56	1.82 ± .37
(+)NH_4^+ (-)C_2H_2	112.07 ± 2.30	24.79 ± 2.79	107.41 ± 5.16	26.09 ± 4.72	127.21 ± 3.98	2.90 ± .47
Sign.	-	*	-	**	-	*
(-)NH_4^+ (+)C_2H_2	36.44 ± 2.46	3.96 ± .65	34.89 ± 3.11	3.66 ± .36	25.77 ± 1.95	1.65 ± .36
(-)NH_4^+ (-)C_2H_2	13.74 ± 2.82	16.29 ± 4.32	17.67 ± 3.30	10.74 ± 3.60	30.60 ± 3.86	1.34 ± .15
Sign.	-	**	-	**	-	NS

† Results expressed as µg N/g dry soil.
†† Results represent the mean of 5 replicates ± SD.
*,** Significantly different at the 5% and 1% probability levels, respectively.
NS Non-significant at the 5% probability levels.

identical to the controls (no urea added) (Figure 2). In contrast, soil NO_3^--N levels rose in urea-treated sod not exposed to C_2H_2 indicating that nitrification was occurring (Figure 2). Nitrate levels were at a maximum after seven days and then decreased slightly most likely as a result of plant uptake. Plant uptake of NO_3^--N was assumed to be occurring in these samples because the KNO_3-treated sod had a gradual decrease in soil NO_3^--N concentration over the course of the nine day study. Denitrification was not considered to be the cause of NO_3^--N loss from the soil because soil moisture content in these sod samples was only slightly greater than 50% of saturation.

As a result of the influence of C_2H_2 on nitrification in these sod samples, it appears to be impractical to utilize the C_2H_2 blockage technique for denitrification studies involving N fertilizers which must be nitrified. Such problems could be avoided by allowing nitrification to occur prior to C_2H_2 usage or by using nitrate-N fertilizers.

Acetylene Disappearance in Soil

Acetylene disappearance occurred at all three soil moisture levels tested in this turf soil. Chow's Procedure (16) showed no significant influence of NH_4^+-N

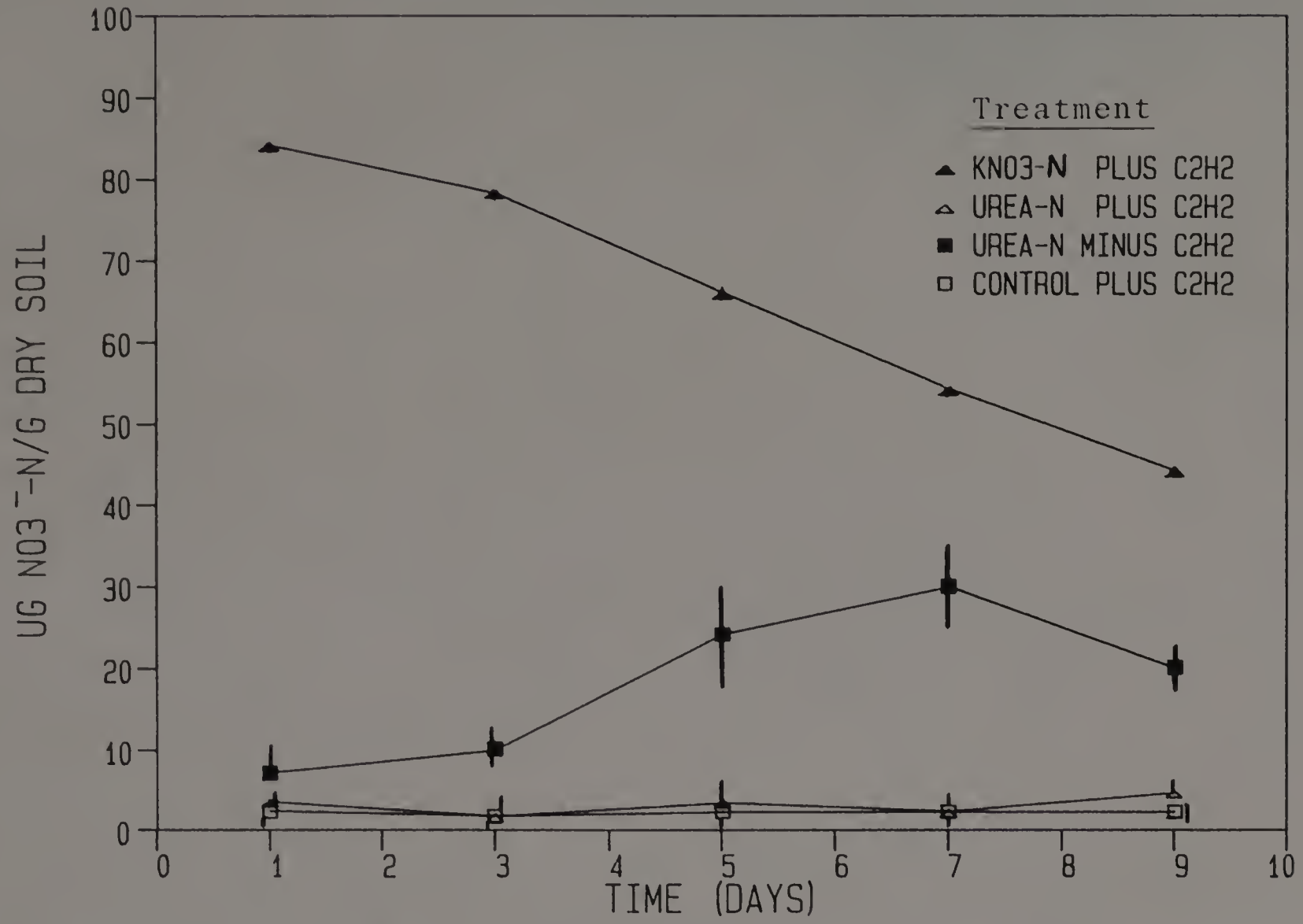

Figure 2. Changes in soil NO_3^--N levels in Kentucky bluegrass sod on a silt soil (75% saturated; 22°C) in the presence or absence of C_2H_2 (1% v/v) following N fertilizer addition (4886 mg N/m^2). Controls had no N applied. Vertical lines represent standard error.

on C_2H_2 disappearance rate at any moisture level. The combined results of the effects of each treatment, with and without NH_4^+-N, on C_2H_2 disappearance are shown in Figure 3. Soils at 50% and 70% of saturation were essentially the same for C_2H_2 disappearance with a lag phase of about four days before large decreases in C_2H_2 levels occurred. This lag phase was extended to six days when the soil was at 90% of saturation. Time for complete acetylene disappearance was also increased by over two days.

Lag phases before C_2H_2 disappearance have been observed by other researchers (60,66). Extended lag-time periods may allow C_2H_2 decomposers to proliferate and/or to switch to a proper metabolic state for C_2H_2 decomposition. Watanabe and de Guzman (66) found fermentative products to be the end result of C_2H_2 decomposition while ethylene formation could not account for the quantity of C_2H_2 which disappeared. A qualitative analysis for ethylene formation in this turf soil showed it to be absent except in a small number of tubes where concentrations were less than 3 ppm. The presence of some ethylene was probably the result of nitrogenase activity associated with free-living dinitrogen fixers in the soil (66).

Although C_2H_2 disappearance was completed within 10

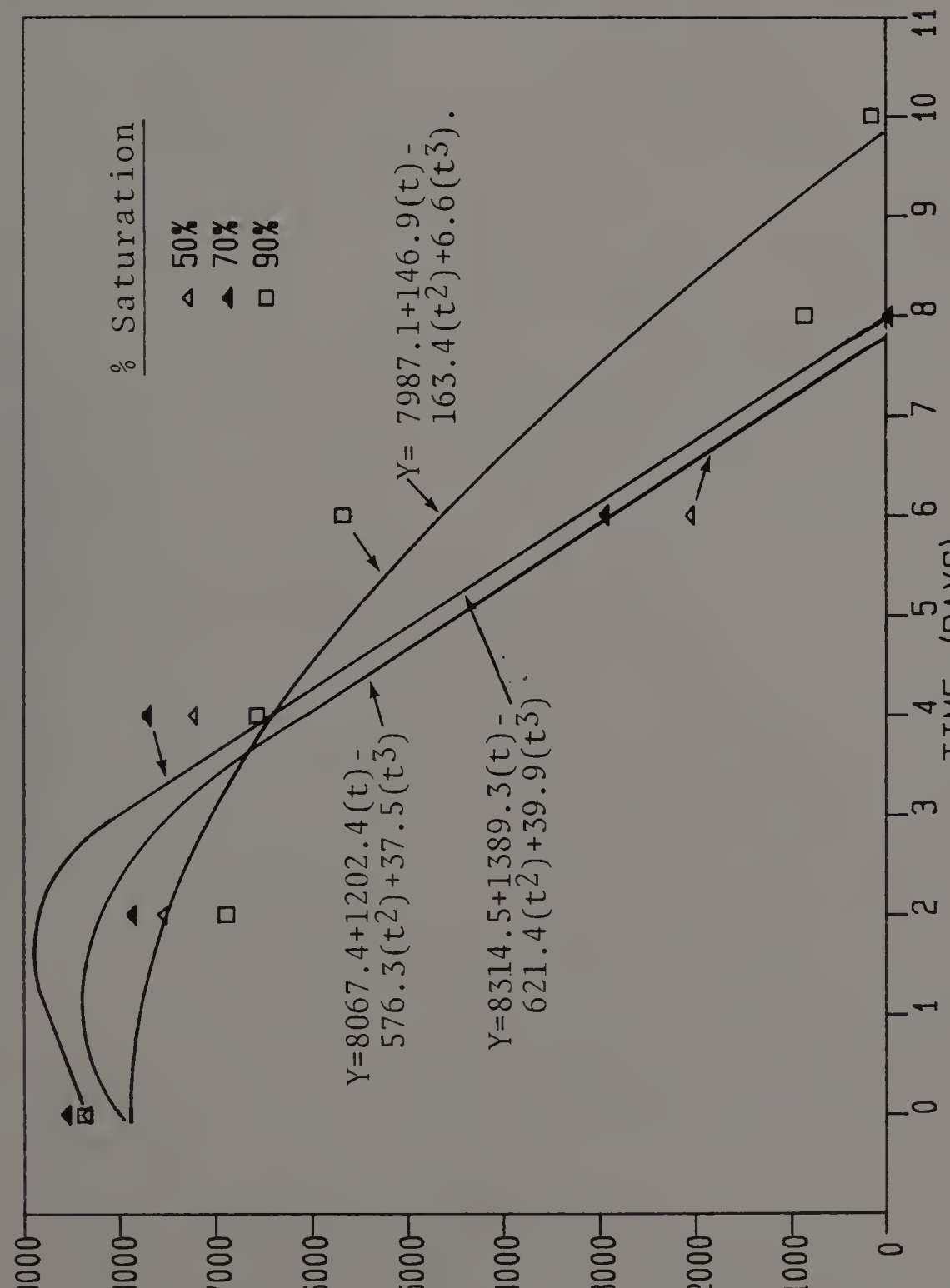

Figure 3. Disappearance of C_2H_2 in the presence of a silt soil (22°C) at three soil moisture levels. Regression analysis lines significant at the 1% probability level. Time = t.

days, it appears that a higher degree of soil saturation may delay or inhibit this process. In fact, anaerobic soil conditions blocked this process completely in the turf soil used in this study (Table 6). These results agree with the work of Terry and Duxbury (60) who did not find C_2H_2 disappearance to occur in aerobic soils made completely anaerobic. Soil microbes in naturally anaerobic soils are known to decompose acetylene rapidly (21).

Acetylene may serve as either a source of energy for soil microbes or be assimilated for the formation of new cell material (21,23,60,66,69). A number of factors influence the decomposition of carbonaceous materials in soil including temperature, pH, O_2 supply, inorganic nutrients and the C:N ratio. Nitrogen is a key nutrient source for microbial growth. In general, carbonaceous material is decomposed slowly when soil N is limiting. Added N stimulates this decomposition. Although C_2H_2 is found to be microbially decomposed in soils, some researchers have shown NH_4^+-N to stimulate this process (60). The added N would lower the wide C:N ratio of the soil and thus speed decay of C_2H_2. This study did not show a stimulation of C_2H_2 disappearance in NH_4^+-N treated soil (Figure 3). Such results agree with Watanabe and de Guzman (66) who found C_2H_2 disappearance to be the same for

Table 6. C_2H_2 levels above a saturated silt soil following a ten day incubation period*.

Soil	C_2H_2 (ppm)
Sterile	9056 ± 79†
Unsterile	8847 ± 337

NS

* Incubation in the dark at 25° C.
† Mean of 4 replicates ± SE.
NS Non-significant at the 5% probability level.

NH_4^+-N treated and untreated soil. Terry and Duxbury (60) may have observed the stimulation of C_2H_2 decomposition because inorganic N levels in their Collamer silty clay may have been low prior to N addition. The authors provide information which shows a Total C:Total N ratio of approximately 9:1. Watanabe and de Guzman (66) also fail to provide this information. The turf soil used in this study had exchangeable NH_4^+-N levels as high as 36 ug NH_4^+-N/g soil when nitrification was inhibited by C_2H_2. Under these conditions a very low C:N ratio may have existed so that the addition of inorganic N did not promote increased decomposition of carbonaceous materials.

Watanabe and de Guzman (66) found complete inhibition of C_2H_2 disappearance in soil when NO_3^--N was applied at a rate of 100 ug N/g soil. Terry and Duxbury (60) found a 55% reduction in C_2H_2 decomposition at this rate and a 98% reduction at 1000 ug NO_3^--N/g soil when compared to soils amended with 10 ug NO_3^--N/g soil. At 100 ug NO_3^--N/g soil C_2H_2 decomposition was inhibited in the turf soil used in this study (Table 7). However, at 50 ug NO_3^--N/g soil 41.6% of the C_2H_2 remained in the tubes after the ten day incubation period. So far, no hypothesis has been offered as to why nitrate-N inhibits acetylene disappearance.

Table 7. Effect of nitrate on C_2H_2 levels above a silt soil after a 10 day incubation period† at 70% of saturation (48.4% H_2O, wt/wt).

µg NO_3-N/g Soil	C_2H_2 (ppm) Sterile Control	C_2H_2 (ppm) Unsterile Soil	F-Test Significance	% C_2H_2 Remaining
100	8335 ± 262††	7630 ± 685†††	NS	91.5
50	8420 ± 320	3500 ± 236	**	41.6
0	8237 ± 237	56 ± 28	***	0.7

† Incubation in the dark at 25°C.

†† Mean of five replications ± standard deviation.

††† $C_2H_2 = -58.3 + 75.7(NO_3^- - \overline{N})$. Regression equation significant at the .01% probability level (r = .99).

*** } Significant difference between pairs at 0.1% and 1.0% probability level.
** }

NS Non-significant at the 5% probability level.

Although C_2H_2 proved to be an effective inhibitor of N_2O reductase it cannot be used indiscriminately without evaluating its influence on other soil microbial processes. Acetylene completely inhibited nitrification in the turf soil used in this study even though sod samples were relatively large. Therefore, the use of C_2H_2 to evaluate denitrification losses from other than NO_3^--N fertilizers must not be carried out unless nitrification is first allowed to occur. Increased soil microbial respiration may occur as a result of C_2H_2 decomposition after about four days of exposure. However, this may be inhibited by the higher soil moisture contents (>70% saturation) and NO_3^--N levels (>50 ug N/g soil) generally associated with denitrification studies. The use of NH_4^+-N does not pose a problem by stimulating C_2H_2 disappearance in this turf soil.

C H A P T E R IV

DENITRIFICATION LOSSES FROM TURF

Introduction

Soil moisture and soil texture influence denitrification by effecting gas exchange and the diffusion of O_2 to active microbial sites (43,50,67). Ryden et al. (50) found that the magnitude of denitrification loss was dependent upon irrigation events. Peak effluxes (28 to 40 mg N/m^2/day) occurred 24 to 36 hours following each event when soil moisture levels were greater than 40% of saturation. These effluxes were short-lived but significant in terms of their contribution to total annual gaseous N losses. Such results concur with work by Sextone et al. (51) in which up to 55% of total denitrification losses occurred within 48 hours following rainfall levels greater than 1 cm. Total N losses were equivalent to 1.1 and 3.2 mg N/kg soil for a sandy loam and clay loam, respectively. A small water input into a finer textured soil may result in greater denitrification losses than from a coarser soil as a result of impedance of gas exchange (67). Less frequent, heavier irrigations could result in lower denitrification losses than more frequent, lighter irrigations because less frequently irrigated soils become dry and applied

water is rapidly redistributed after irrigation (43).

Denmead et al. (24) reported significant N_2O-N emissions (38 mg N/m^2) from the second to fifth day following the flooding of fertilized rice fields. The N_2O-N losses observed from this red clay represented 81% of the total N_2O-N loss observed during an eighteen day experimental period. In another study, Ryden (46) found 61% of the annual denitrification losses (2,900 mg N/m^2) to occur within a five week period with peak emissions of 50 mg N/m^2/day. Total losses were equal to 6.6% of the N applied. Wetter soil conditions (greater than 20% wt/wt) and higher soil NO_3^- levels (greater than 5 ug N/g soil) were always associated with increased denitrification losses from perennial ryegrass (Lolium perenne) swards (46). Low soil NO_3^--N levels (less than 0.5 ug N/g soil) resulted in reduced annual denitrification losses (17 mg N/m^2) even following high urea-N applications (180 kg N/ha) and flooded soil conditions (52).

Soil nitrate-N levels are not the only factor influencing denitrification. In fact some research has shown no association between NO_3^--N levels and denitrification events (48,50). Vinther (62) found equal losses from two fallow fields even though soil NO_3^--N levels were different. It was concluded that soil O_2 tension and % organic matter also influence

denitrification losses and may override the effects of soil nitrate-N levels.

The presence of actively growing plants may stimulate soil denitrification by as much as five-fold (17,58,62). Plant roots may contribute to the biological oxygen demand of the soil as a result of root respiration and may also contribute to the soil's organic matter content thereby stimulating microbial respiration. However, the ability of plant roots to quickly reduce soil NO_3^--N may counteract this rhizosphere effect (46). Denitrification losses from some cropped fields have been reported to be less than losses from fallow fields (3,27). Losses from soils under grass crops are typically much lower than losses from soils under vegetable crops which have less dense root systems despite similarities in soil types and amounts of N fertilizer applied annually (3,18,46,48,62). Although the denitrification efflux may be similar in magnitude, the duration is shorter in the presence of grasses due to rapidly lowered soil NO_3^--N levels (46). Losses from irrigated vegetables (lettuce, celery, broccoli and artichokes) were as high as 233 kg N/ha/year (46,48) amounting to as much as 52% of the applied N. In comparison, losses from annual grasses (wheat, barley and rice) ranged from less than 1 to 19 kg N/ha/year (3,18,62). Denitrification losses from

perennial ryegrass ranged from 11.1 to 29.1 kg N/ha/year (4.4 to 6.6% of applied N) even after ammonium nitrate applications as high as 500 kg N/ha/year (46).

Denitrification rates have been strongly correlated with the amount of readily available soil organic matter (7,12,57). Soils receiving organic amendments had higher rates of denitrification which were associated with decreased soil O_2 levels resulting from increased microbial respiration (43). Bremner and Shaw (11) found additions of glucose and cellulose to rapidly stimulate denitrification in comparison to lignin and sawdust. In contrast, other researchers have found that the addition of an organic substrate such as glucose decreased denitrification (10) because the increased soil C:N ratio resulted in the immobilization of nitrogen and made it less available for denitrification.

Many factors influence the quantitative effects of temperature upon denitrification rate. These include the diversity and availability of soil organic substrate and the selective effects of temperature on different bacterial species and denitrifying populations (31,34). A Q_{10} value (change in rate caused by a temperature increase of 10 ^{o}C) of two or three is generally observed when soil temperatures are greater than 15 ^{o}C (17,25) but is larger (up to eleven) with lower soil temperatures (5 to 15 ^{o}C)

(31,46).

Bremner and Shaw (11) found maximum soil denitrification rates to occur in vitro at 30 ^{o}C in six soils amended with glucose and NO_3^--N. This rate remained constant up until 60 ^{o}C with complete inhibition of denitrification at 70 ^{o}C. However, optimum temperatures for denitrification in the field could vary due to differences in soil moisture content, pH, availability of substrate and the species of denitrifying bacteria. Bollag et al. (9) found that four isolated species of soil denitrifying bacteria grew best at 30 ^{o}C (compared with 10, 22 and 37 ^{o}C) while Gamble et al. (32) found a strong correlation between temperatures favoring the growth of certain isolates in vitro and the mean annual temperature of the soil from which the species were isolated. Denitrifiers isolated from temperate soils with a mean annual temperature of less than 20 ^{o}C grew best in vitro at temperatures less than 22 ^{o}C.

The objectives of this study were to determine total denitrification losses from a Kentucky bluegrass sod (Poa pratensis L. var. 'Baron') as influenced by soil texture, soil moisture content, soil temperature and fertilizer N source.

Materials and Methods

Influence of Soil Moisture Content on Denitrification

Nine sod samples from the silt soil site were installed in the acrylic turf chambers (Appendix C). The initial soil moisture content of the underlying soil was 91.7% of soil saturation (48.4% water, wt/wt).

Six sod samples were allowed to air dry until the soil was approximately 50% saturated. Potassium nitrate (3.02 g/sample) was dissolved in 1,500 ml water and applied to two samples while another sample received water only. Final soil moisture content of these sod samples was 100% of saturation. Six hundred ml of water were used to apply KNO_3 to two more samples bringing the soil moisture contents to approximately 75% of saturation. Another sample served as a control (no N-fertilizer applied) at this soil moisture level. The three remaining sod samples were allowed to dry to a soil moisture content of 37% of saturation before 400 ml of water were applied. Two samples received KNO_3 applications. Final soil moisture content was approximately 50% of soil saturation. Acrylic lids were sealed onto the chambers following N-fertilizer and water application and N_2O-N emissions were monitored for ten days (Appendix H).

After ten days the sod samples described above were

returned to the field and the acrylic chambers were filled with nine fresh sod samples from the silt soil site. Initial soil moisture content of this sod was 66% of soil saturation. Potassium nitrate was applied in quantities of water ranging from 400 to2,000 ml with treatments varying by 200 ml increments. Denitrification losses were then monitored from these samples. Final soil moisture contents ranged from 72% to 106% of soil saturation.

The above study was repeated with sod from the silt loam soil site. Initial soil moisture content was 93.5% of saturation (40% water, wt/wt). After soil moisture levels fell to approximately 68% of soil saturation, seven sod samples received KNO_3 (3.02 g/sample) applied in water quantities ranging from 400 to 900 ml. The two remaining sod samples were dried to 45% of soil saturation and fertilizer was applied in 500 ml water. Final soil moisture levels ranged from 33 to 101% of soil saturation.

Both of the above studies were conducted at a soil temperature of 22 (±2) ^{o}C and samples were illuminated with fluorescent lighting (12 hours day/night, 19.1 W/m^2).

Six soil samples were removed from each sod sample using a cork borer (1.2 cm diameter) and exact soil moisture contents were determined gravimetrically following the completion of each study. Final soil NO_3^--N

levels were also determined as described in Chapter 3.

A stepwise-regression analysis was used to test for a significant relationship between total N_2O-N loss and the level of soil saturation.

The Influence of Three Soil Temperatures on Denitrification

Six sod samples from the silt soil site were installed in the acrylic chambers and incubated at 22 ^{o}C (day/night). Initial soil moisture content was 66% of soil saturation. Potassium nitrate (3.02 g/sample) was applied to three sod samples in 600 ml water while controls received water only. Final soil moisture content was approximately 75% of saturation. Soil temperature was monitored in each sample using a soil thermometer (Fisher Scientific, Catalogue # 15-077) which was inserted through a hole in the chamber wall at a soil depth of 5 cm. Daily nitrous oxide-N effluxes were measured and total N_2O-N loss calculated for a ten day period. The study was repeated at 26 ^{o}C and 30 ^{o}C and a linear regression analysis was used to determine the relationship between total nitrous oxide-N losses and soil temperature.

Influence of Increased Soil Temperature and Soil Saturation on Denitrification

Eight sod samples were collected from the silt loam soil site and installed into the acrylic chambers. Initial soil moisture content was 87% of soil saturation. All samples were completely saturated with 2,000 ml of water with four samples receiving KNO_3 (3.02 g/sample). Two fertilized and two unfertilized samples were incubated at a soil temperature of 35 ^{o}C. The remaining four chambers were incubated at 30 ^{o}C. Soil NO_3^--N levels were determined for all samples after nitrous oxide-N effluxes had terminated.

The above experiment was repeated using sod from the silt soil site. Initial soil moisture content of this soil was 79% of saturation.

An ANOVA was used to test for significant differences between total N_2O-N losses within each soil.

Determination of the Limiting Soil Factors Affecting Denitrification

It was observed that N_2O-N effluxes from the turf soils studied generally reached a maximum approximately three days after N-fertilizer application and then declined to 0 mg N_2O-N/m^2 within ten days of incubation. To qualitatively determine which soil factor might become

limiting to the denitrification process during the ten day incubation period, an experiment was designed utilizing the silt loam sod from the previous study. One previously NO_3^--N treated sample from each of the 30 and 35 ^oC treatments again received KNO_3 (3.02 g/sample) delivered in 1,000 ml water. The remaining previously NO_3^--N treated samples received KNO_3 plus a 5% glucose solution delivered in 1,000 ml water. One previously untreated sample from each soil temperature treatment received the 5% glucose solution (1,000 ml). The remaining two chambers received 1,000 ml water and served as controls. Denitrification losses were monitored for 5 days.

Denitrification Loss from Urea-N Applied to Sod

Eight sod samples were collected from the silt soil site and installed into the acrylic chambers. Initial soil moisture content was 70% of saturation. Each sample received 0.97 g urea (4,886 mg N/m^2) in 600 ml water and had a final soil moisture content of approximately 50% of soil saturation. Samples were then saturated at intervals of one, three, five or seven days following urea application. This approach allowed nitrification to occur during unsaturated soil conditions for increasing lengths of time prior to soil saturation. Two replications per treatment were utilized and all samples were incubated at

22 °C. Soil samples were collected just prior to saturation and analyzed for soil nitrate-N.

A stepwise-regression analysis was used to test for a significant relationship between total N_2O-N losses and the time period allowed for nitrification.

Results and Discussion

Soil moisture content had a significant quadratic influence on denitrification loss from sod on both the silt and silt loam soils (Table 8). Total denitrification losses were small when both soils were less than 80% saturated (Figure 4) and represented less than 0.1% and 0.4% of the N applied (4,886 mg N/m^2) to the silt and silt loam soils, respectively (Appendix I). Virtually no nitrous oxide-N losses were measured from controls (no N applied) at soil moisture contents below 80% soil saturation and only 4 mg N/m^2/10 days at 98% of soil saturation (Figure 4).

Denitrification losses increased at soil moisture levels greater than 80% of saturation and maximum losses occurred at saturation (Figure 4). These losses accounted for approximately 5% and 2% of N applied to the silt and silt loam, respectively (Appendix I). These losses are within the range (0.1% to 6.6% of applied N) observed by

Table 8. Regression of % soil saturation on total denitrification losses (mg N_2O-N/10day/m^2) from Kentucky bluegrass sod after NO_3^--N addition (4886 mg N/m^2).

Soil Type	Dependent Variable	Independent Variable†	R^{2}‡	F-test
silt	mg N_2O-N/10day/m^2	= 1432.50-38.96(SAT)+0.26(SAT^2)	0.94	**
silt loam	mg N_2O-N/10day/m^2	= 130.80-5.40(SAT)+0.05(SAT^2)	0.97	**

† SAT = %soil saturation.
‡ R^2 = coefficient of multiple correlation squared.
** Regression equation significant at the 1% probability level.

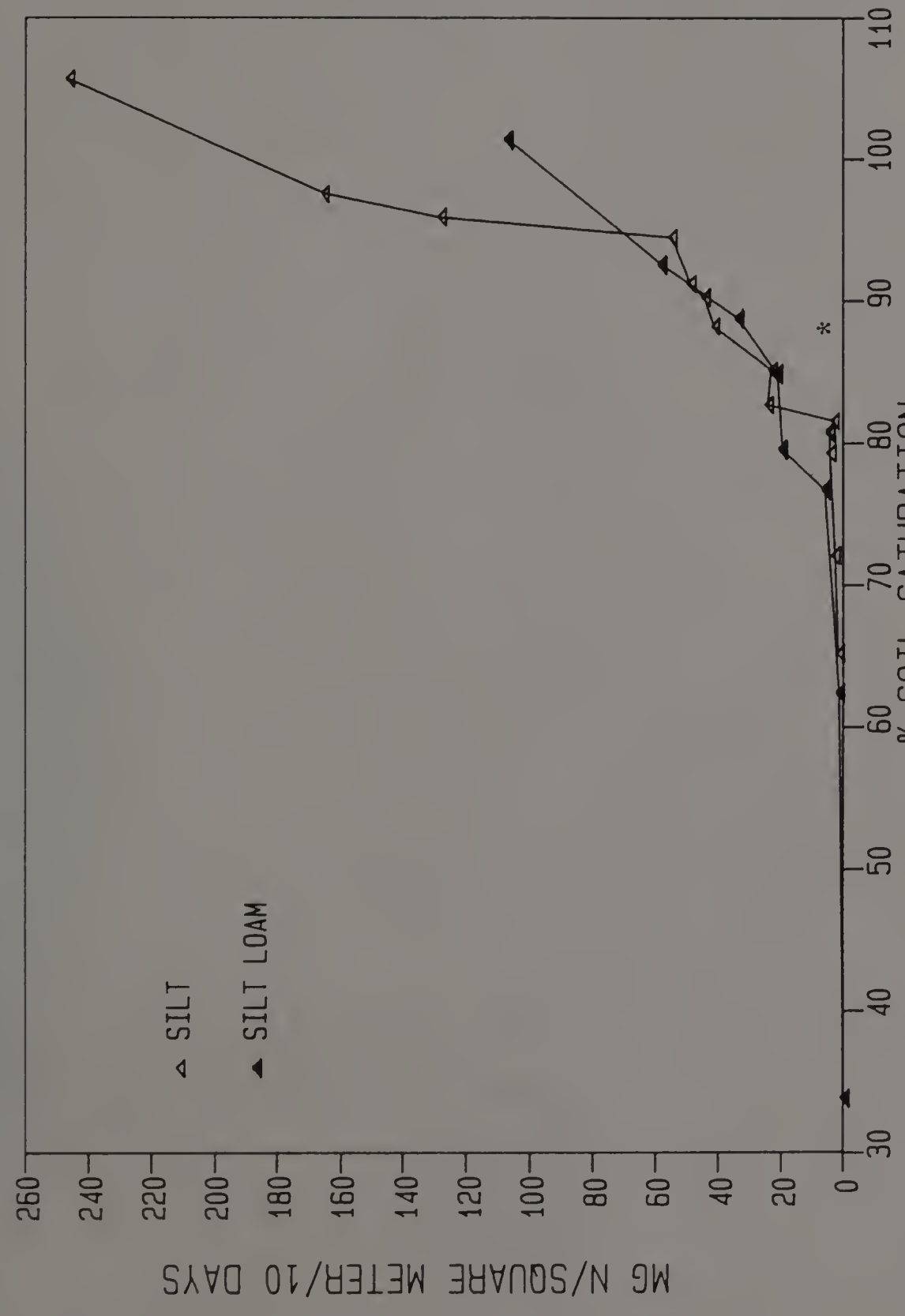

Figure 4. The influence of soil moisture content on total denitrification losses from Kentucky bluegrass sod on two soil types incubated at 22°C following KNO_3—N addition (4886 mg N/m^2). Control(no N applied)=*.

other researchers working with permanent grass swards (43,46). Smith and Tiedje (55) also found denitrifying activity to be greatly increased as soil moisture levels increased from 75% of saturation. Sextone et al. (51) reported soil moisture contents of 71% of saturation in a clay loam soil and 93% of saturation in a sandy loam soil to stimulate maximum denitrification losses.

Soil texture can influence the extent of denitrification since water movement through a finer textured soil may be impeded resulting in temporary anaerobic soil conditions (67). Sextone et al. (51) reported that greatest denitrification losses occurred in a clay loam and sandy loam soil at 71% and 93% of saturation, respectively. Lower soil moisture levels resulted in greater denitrification losses from the finer textured soil because of the clay loam's slower water infiltration rate and larger water holding capacity. This resulted in a greater and more prolonged impedance of O_2 gas exchange. Because of the design of the acrylic turf chambers (Appendix A), water drainage was not possible from the sod samples. Therefore, the difference in soil moisture content as affected by a soil's ability to drain was not as great a variable in this study as it was in the field studies. In addition, soil NO_3^--N content was not a variable in this study because it was in the same range

(50-70 ug N/g soil) for both soils at the time of the completion of the study. Therefore, the larger denitrification losses associated with the silt soil may have been due to other soil properties, including pH and % organic matter content (Table 1) as well as the silt soil's ability to support larger denitrifying populations (Table 3).

Large percentages of the total denitrification losses occurred during a 2 to 5 day period (Figures 5 to 8) for both soils with a longer duration of the N_2O-N efflux at the higher soil moisture levels and for the silt soil. Under completely saturated soil conditions 80% of the total denitrification loss occurred from day two to four in the silt loam soil (Figure 5). Sixty four percent of the total losses also occurred during this time period at a soil moisture content of 80% of saturation (Figure 6). Similar results were obtained from the silt soil where 81% of the total denitrification losses occurred during the first five days of saturation (Figure 7). Large, but short-lived N_2O-N emissions have been observed by other researchers (24,46,50,51) with peak emissions as high as 50 and 90 mg N/m^2/day from perennial ryegrass swards. Peak emissions were as high as 48 and 60 mg N/m^2/day for the silt loam and silt turf soils, respectively (Figures 5 and 7).

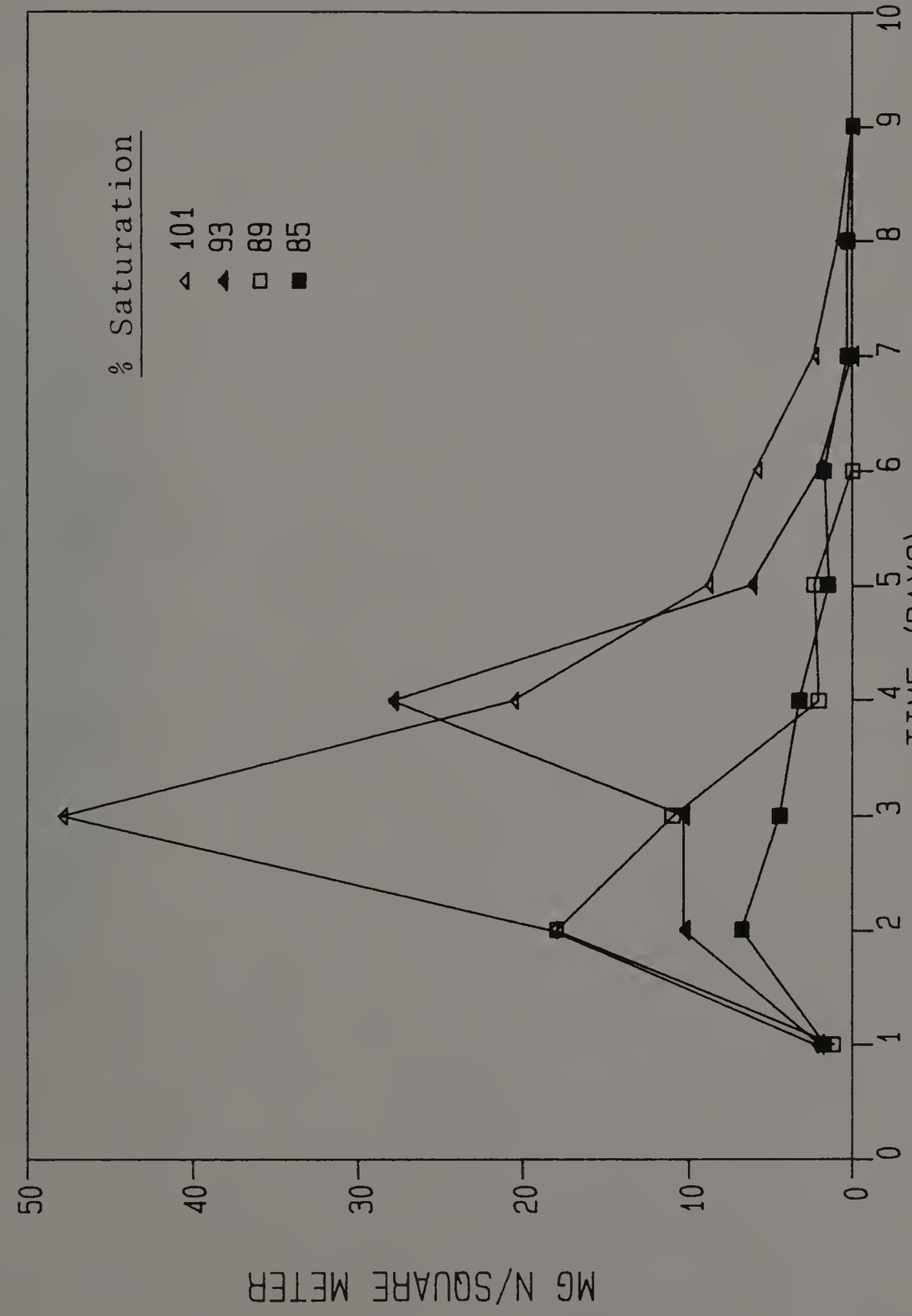

Figure 5. N_2O-N efflux from Kentucky bluegrass sod on a silt loam soil (22°C) at soil moisture levels >80% of saturation following KNO_3—N addition (4886 mg N/m^2).

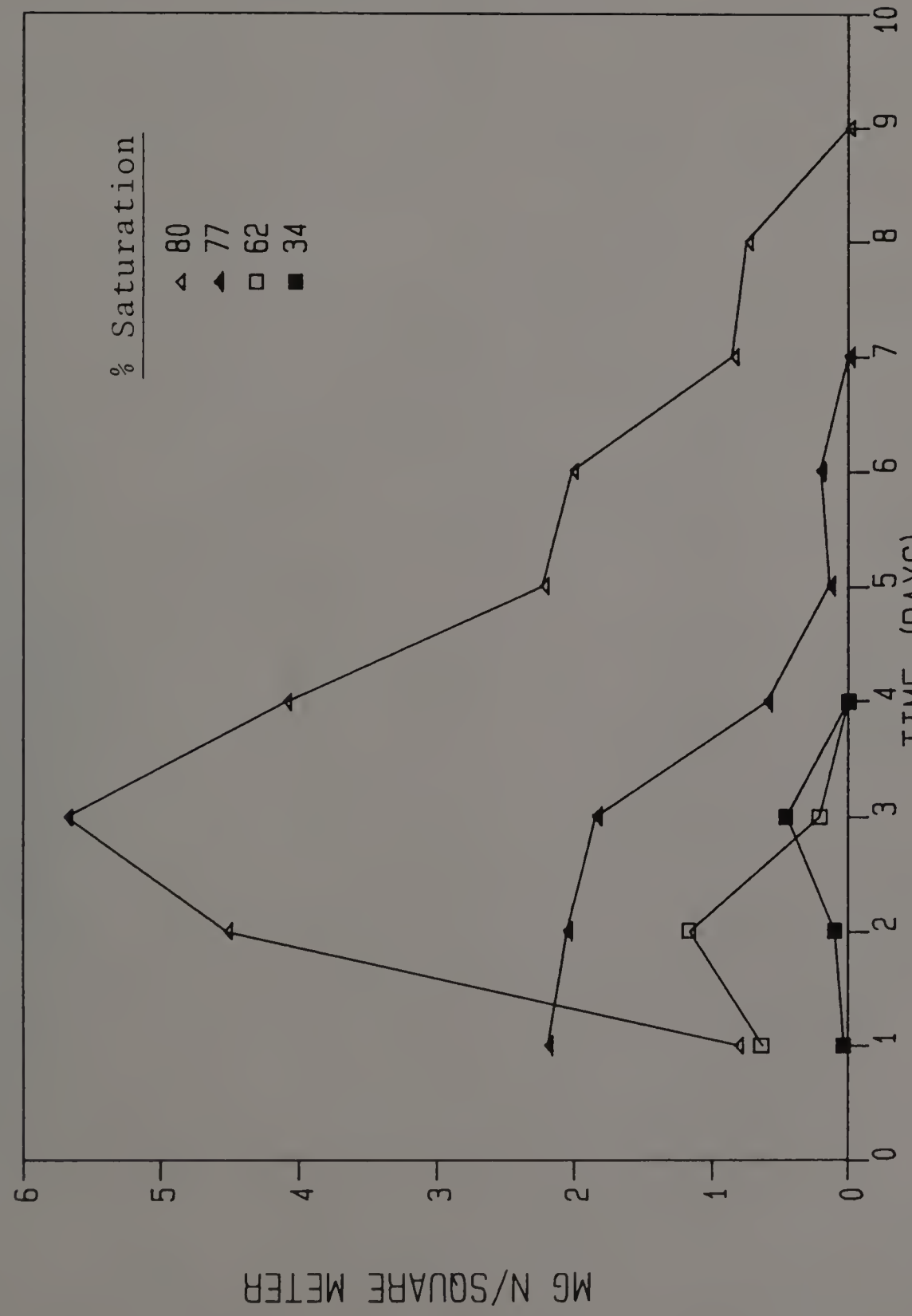

Figure 6. N_2O-N efflux from Kentucky bluegrass sod on a silt loam soil (22°C) at soil moisture levels ≤80% of saturation following KNO_3 – N addition (4886 mg N/m^2).

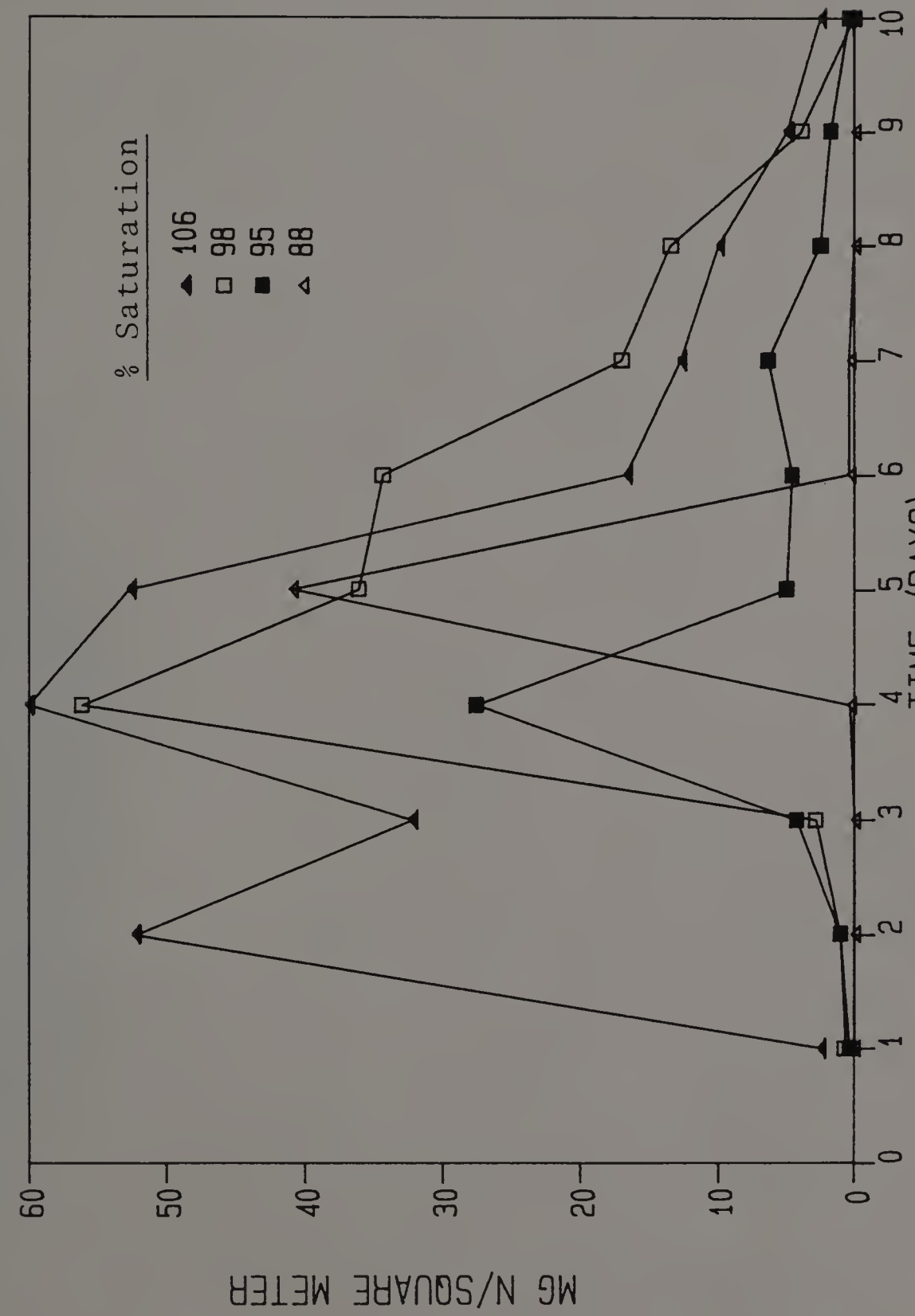

Figure 7. N_2O-N efflux from Kentucky bluegrass sod on a silt soil (22°C) at soil moisture levels >85% of saturation following KNO_3-N addition (4886 mg N/m^2).

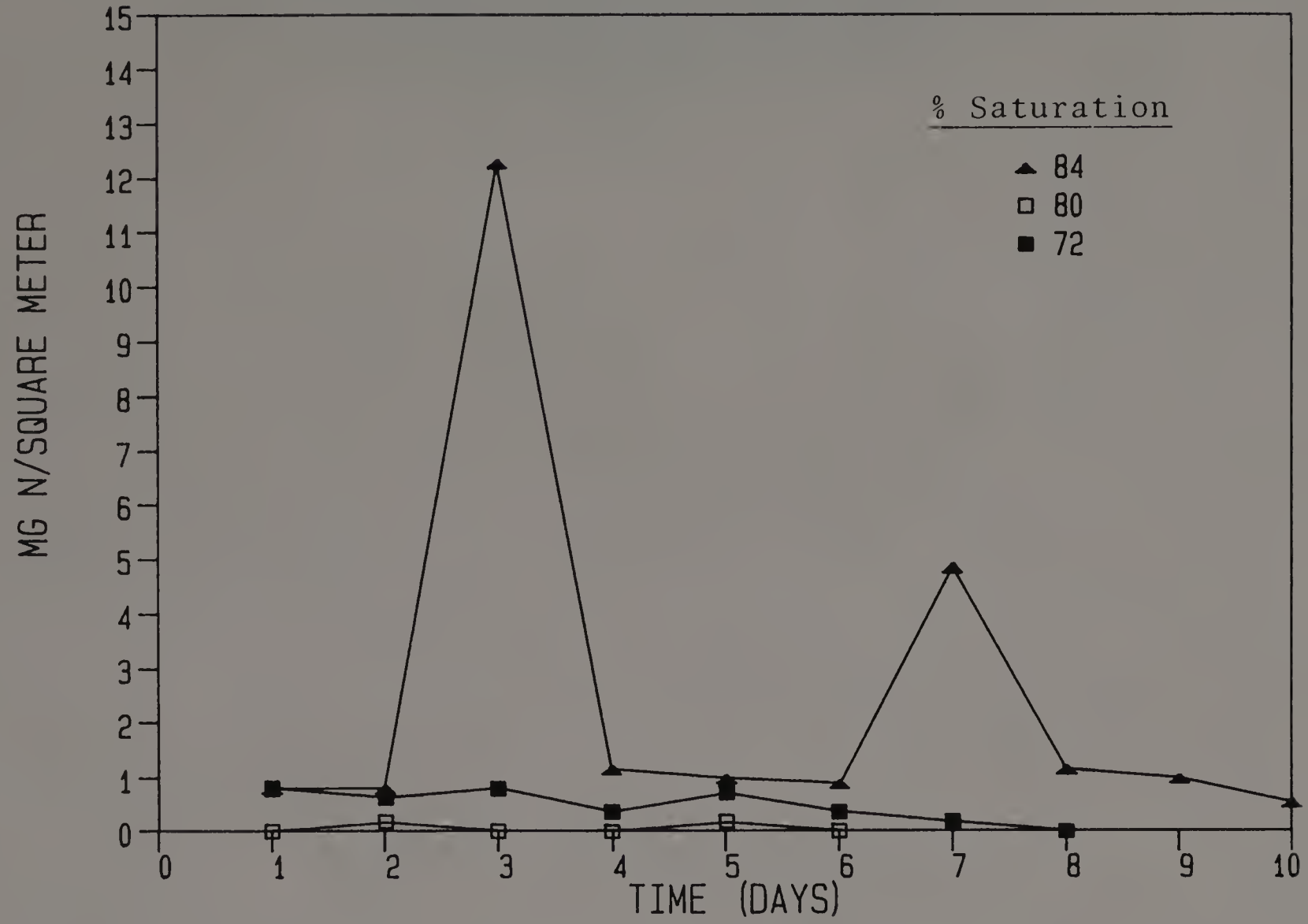

Figure 8. N_2O-N efflux from Kentucky bluegrass sod on a silt soil (22°C) at soil moisture levels ‹85% of saturation following KNO_3 – N addition (4886 mg N/m^2).

A soil moisture content of approximately 75% of saturation was selected to examine the influence of soil temperature on denitrification losses. Soil moisture levels below 80% of saturation were found to result in denitrification losses which did not vary much as a result of small differences in moisture content (Figure 4). The silt soil was also selected because of its larger denitrification potential, as indicated by actual denitrification losses under saturated soil conditions (Figure 4) and large denitrifier populations (Table 3).

The magnitude of N_2O-N efflux increased as soil temperature increased from 22 to 30 ^{o}C (Figure 9). Total denitrification losses were linearly related to soil temperature (Table 9). The loss observed from the sod soil at 22 ^{o}C was in close agreement with the losses observed in the previous study where the silt soil moisture level was approximately 75% of saturation and soil temperature was 22 ^{o}C (Figure 4 and Appendix I). These losses represented less than 0.1% of the N fertilizer applied.

A calculated Q_{10} value for the data in Table 9 is 5.4. This is high in comparison with the Q_{10} value of two or three observed in other soils (17,25). However, variation from this value may occur because Q_{10} is influenced by many soil factors such as availability of organic substrate, species of denitrifying bacteria and

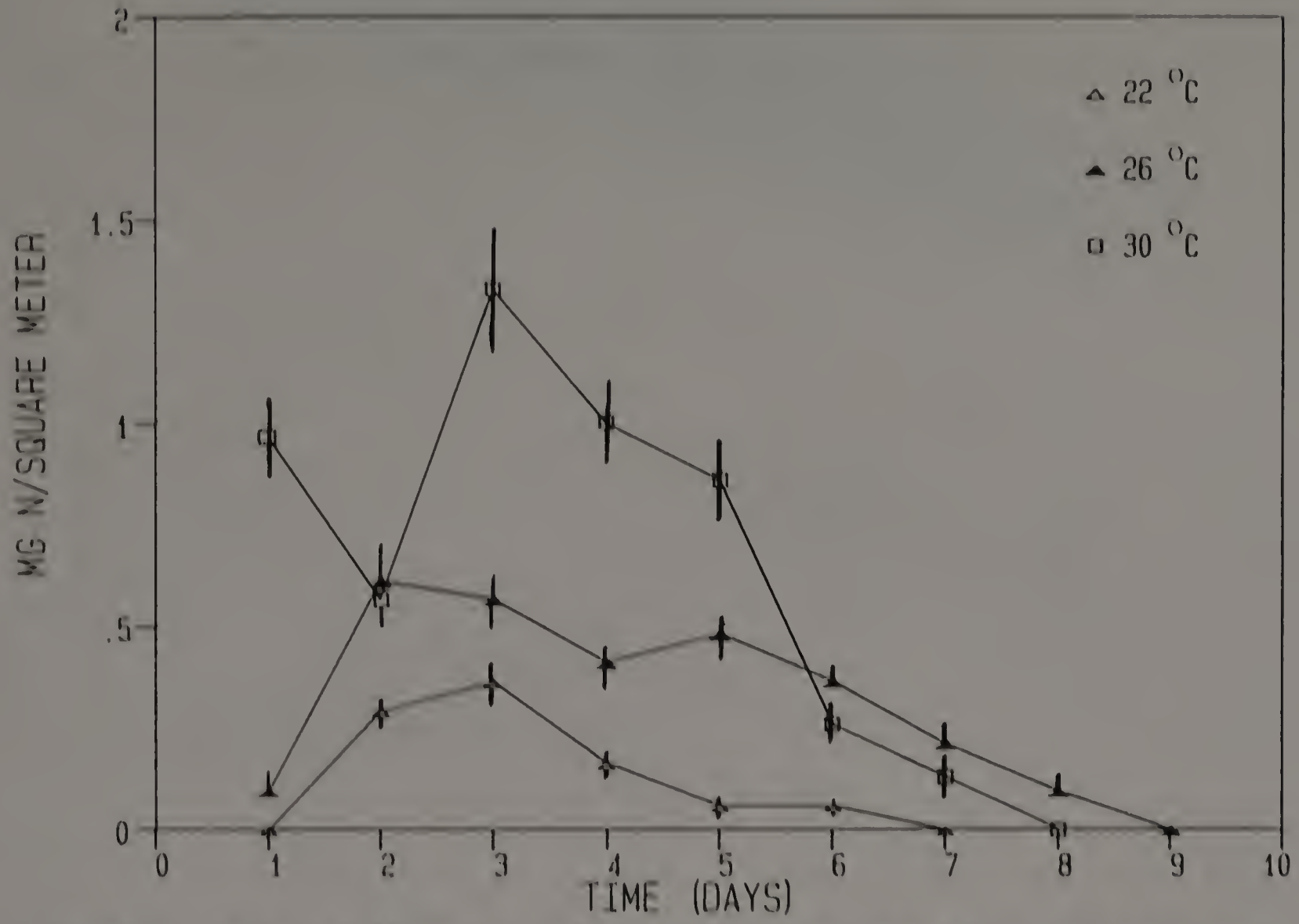

Figure 9. N_2O-N efflux from Kentucky bluegrass sod on a silt soil (75% saturated) incubated at three soil temperatures following KNO_3 - N addition (4886 mg N/m^2). Vertical lines represent standard error.

Table 9. Total denitrification losses from Kentucky bluegrass sod on a silt soil (75% saturated) incubated at three soil temperatures after NO_3^--N addition (4886 mg N/m^2).

Soil Temperature (°C)	mg N_2O-N/10day/m^2 †	% Applied N Lost
22	0.98 ± 0.17‡	.02
26	2.93 ± 0.39	.06
30	4.98 ± 0.51	.10

† mg N_2O-N/10day/m^2 = 0.49(°C) - 9.70; r^2 = .99** (P=.01).
‡ Mean of three replications ± standard error.

degree of soil saturation (31,34).

The interaction of increased soil temperatures and saturation resulted in comparatively large denitrification losses from both soils (Table 10). These losses were equivalent to approximately 40 and 80% of N applied to the silt loam and silt soil, respectively. Ryden (46) found the largest denitrification losses to occur from a perennial ryegrass sward when soil moisture levels and temperatures increased in the upper 2.5 cm of soil. This has also been observed by others working with grass swards (17,44).

Saturated turf soil conditions and increased soil temperatures resulted in denitrification losses which were much higher than the losses reported by others working with perennial grasses (42,44,46). Peak N_2O-N emissions (Figures 10 and 11) from both turf soils are also considered to be extremely high in comparison with peak N_2O-N emissions from other fertilized grass swards (17,42). Ryden (48) did, however, observe a peak flux of 3,360 mg N/m^2 from irrigated vegetables. It is evident that very large N fertilizer losses may occur in a short period of time as a result of denitrification in soils with environmental conditions favoring this process.

Greater denitrification losses did not result by increasing the soil temperature from 30 to 35 ^{o}C (Table

Table 10. Total denitrification losses from Kentucky bluegrass sod on two soil types at 100% soil saturation and incubated at high soil temperatures after NO_3^--N addition (4886 mg N/m^2).

Soil Type	Soil Temperature (°C)	mg N_2O-N/10day/m^2	% Applied N Lost	F-test
silt	30	4217±10†	86	NS
	35	4102±32	84	
silt loam	30	2037±13	42	NS
	35	1866±13	38	

† Mean of three replications ± standard error.
NS Non-significant at the 5% probability level.

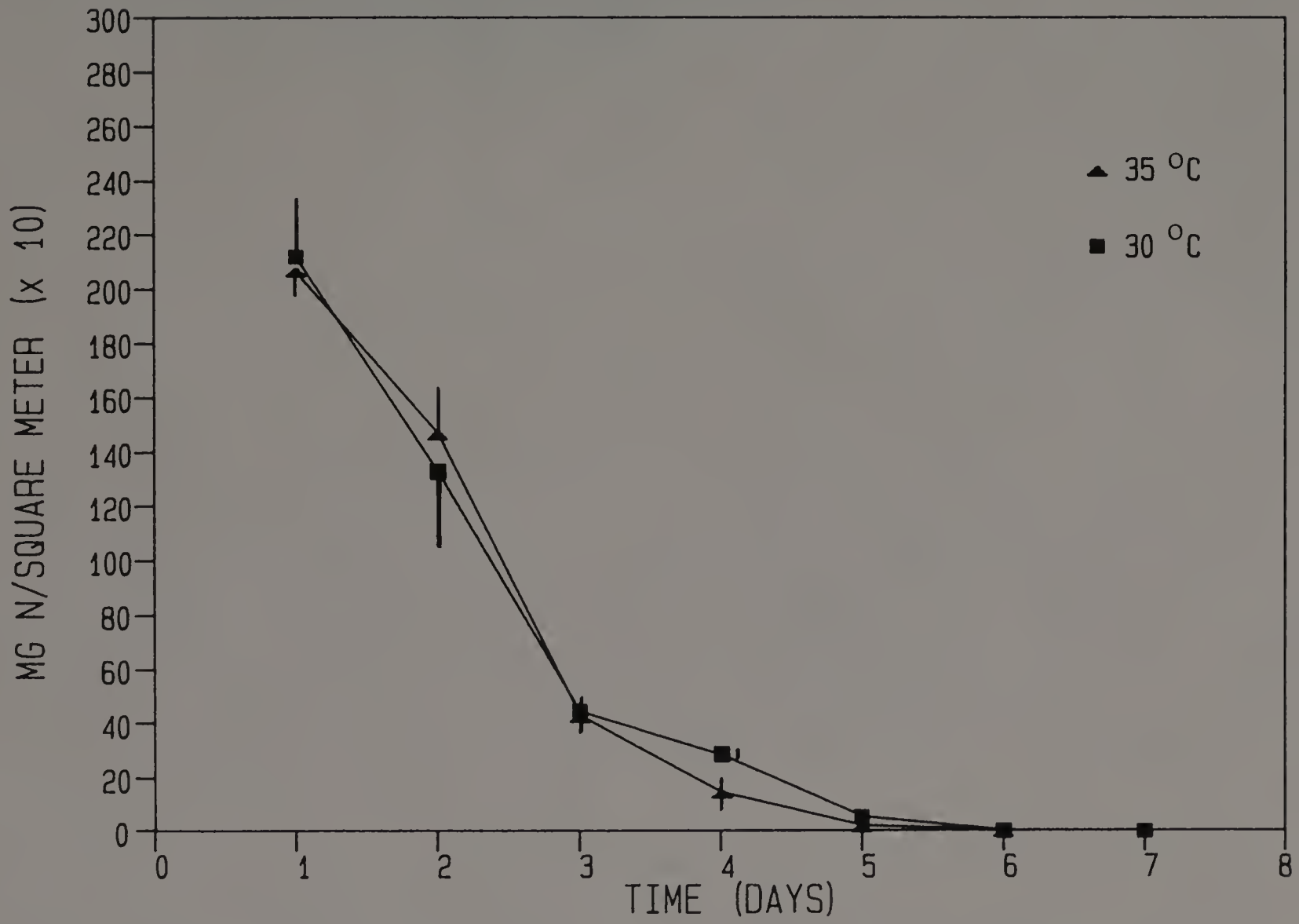

Figure 10. N_2O-N efflux from Kentucky bluegrass sod on a saturated silt soil incubated at high soil temperatures following KNO_3-N addition (4886 mg N/m^2). Vertical lines represent standard error.

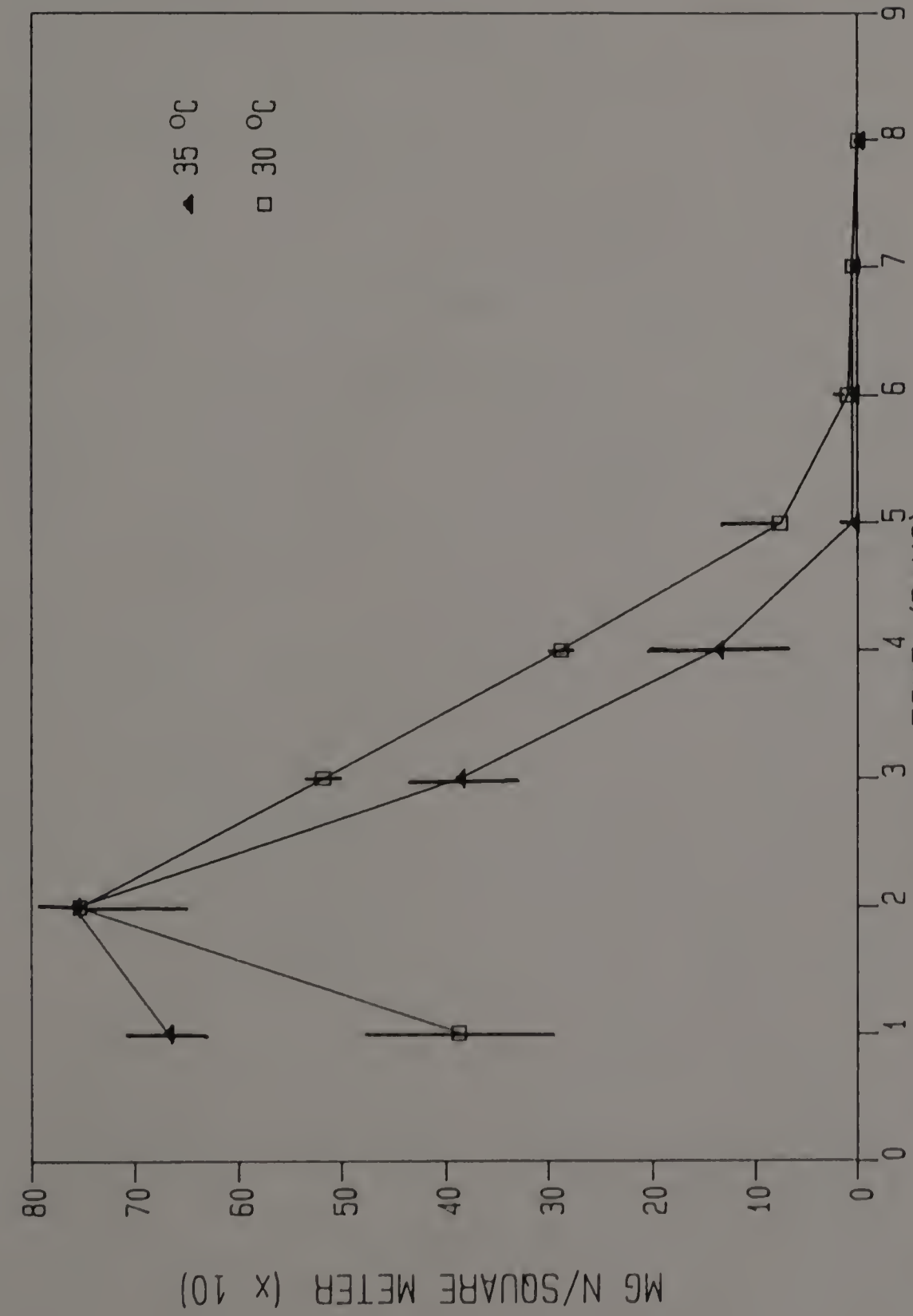

Figure 11. N_2O-N efflux from Kentucky bluegrass sod on a saturated silt loam soil incubated at high soil temperatures following KNO_3-N addition (4886 mg N/m^2). Vertical lines represent standard error.

10) which may indicate that a maximum denitrification rate occurred at approximately 30 ^{o}C. Therefore, under our imposed experimental conditions, increasing soil temperatures above 30 ^{o}C would not result in increased denitrification losses.

Final soil nitrate-N levels and denitrification losses accounted for a large percentage of the applied N (Table 11) in this study. Although variation existed for NO_3^--N in the silt loam soil, accountability for the fate of applied N was still acceptable.

Ryden (46) found denitrification losses from grass swards to be stimulated when NO_3^--N was greater than 5 ug N/g soil. Yet denitrification in our soils was found to decline to essentially 0 mg N/m^2/day (Figures 5, 9 and 11) despite continued saturated soil conditions and elevated NO_3^--N levels (range 7 to 70 ug N/g soil). Ryden (44) found that it was the soil moisture content of the upper 2.5 cm of the soil profile below a ryegrass sward which was associated with the majority of the N_2O-N losses. This zone may also be where the majority of denitrification losses occur from our sod. A closer examination of the distribution of soil water within our saturated sod samples was attempted. However, the sampling of soil for the purpose of determining soil moisture levels at different depths was found to be

Table 11. Final NO_3^--N levels and total denitrification losses from Kentucky bluegrass sod on two soil types following the addition of NO_3^--N*.

Soil Type	NO_3^--N (ppm)	NO_3^--N (mg N/m^2)	Denitrification Loss (mg N_2O-N/10day/m^2)	% Applied N
silt	7.4 ± 1.6	525.5 ± 113.6	4160 ± 81	96
silt loam	20.7 ± 15.2	1470.0 ± 1079.4	1951 ± 121	70

* 4886 mg N/m^2

impossible using either a cork borer (1.9 cm diameter) or a knife to remove samples because any pressure applied to the sod's surface resulted in compression of the underlying soil. Standing water could be observed in the bottom 2.5 cm of the clear turf chambers under extremely saturated conditions. Theoretically, a redistribution of soil water within the sod would result in a redistribution of soil NO_3^--N and, therefore, soil water or NO_3^--N would become the limiting factors in denitrification. As such, nitrate-N level and/or soil water level in the uppermost few centimeters of soil was believed to be the limiting factor in denitrification in our sod.

A second application of nitrate-N to the sod resulted in the restimulation of denitrification while the addition of water alone did not (Figure 12). The water application brought the standing water level in the chambers to within the top 2.5 cm of the soil profile. Denitrification losses did not decline even after five days. These results strongly suggest that the NO_3^--N level in the uppermost portion of the sod was critical to the denitrification process. The addition of a 5% glucose solution had essentially no effect on the restimulation of denitrification indicating that organic carbon was not limiting (Figure 12).

Yeomans and Beauchamp (68) reported an

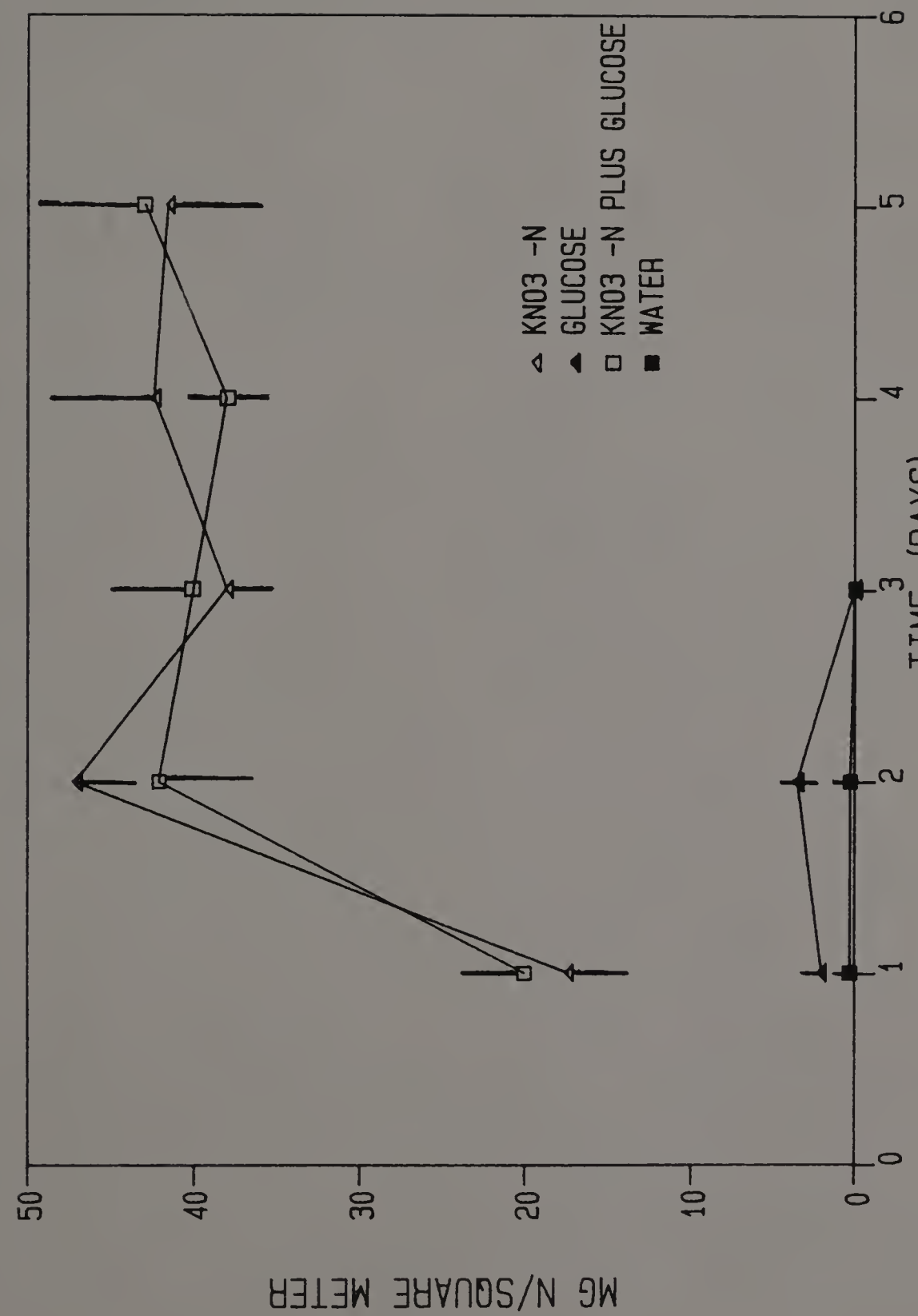

Figure 12. N_2O-N efflux from Kentucky bluegrass sod on a saturated silt loam soil (30°C) following the addition of either one liter of a KNO_3-N solution (3.02 g/l), a glucose solution (5% wt/wt), a KNO_3-N plus glucose solution, or water alone.

acclimatization of soil denitrifiers to C_2H_2 (0.1 to 1.0%, v/v) after seven days of exposure which resulted in complete ineffectiveness of the acetylene inhibition technique. In contrast, this was not found to occur in the silt loam turf soil since the addition of NO_3^--N resulted in continued N_2O-N production even after a previous exposure of the soil to acetylene (1%, v/v) for ten days (Figure 12).

Urea is an important fertilizer used by the turf industry. It serves as a high and relatively inexpensive N source which may be easily applied in liquid or solid form. Urea hydrolysis results in the production of NH_4^+-N which may be nitrified to NO_3^--N. The NO_3^--N is then subject to plant and microbial uptake, leaching or denitrification.

Denitrification loss from urea applied to the silt soil was greatest when the soil was saturated following five days of nitrifying soil conditions in which soils were 50% saturated (Table 12). Peak losses corresponded with peak soil NO_3^--N levels (r=0.93). These losses were approximately equal to the losses occurring from the saturated silt soil at 22 ^{o}C following KNO_3 addition (Figure 4). Denitrification losses from urea following seven days of nitrification were lower than losses following five days of nitrification (Table 12). Soil

Table 12. Total denitrification losses from Kentucky bluegrass sod on a saturated silt soil (22°C) following the nitrification of urea-N addition (4886 mg N/m^2)

Time of Nitrification† (days)	mg N_2O-N/10day/m^2	ug NO_3^--N/g soil	% Applied N Lost
1	38.6 ± 3.8‡‡ ‡‡‡	9.9 ± 1.4	0.8
3	120.9 ± 11.7	13.1 ± 2.6	2.5
5	270.4 ± 31.9	16.6 ± 2.1	5.5
7	204.7 ± 41.5	14.4 ± 1.8	4.2

† Nitrification occurred at a soil moisture content of 50% of saturation.
‡‡ Mean of two replications ± standard error.
‡‡‡ mg N_2O-N/10day/m^2 = -61.8 + 101.6(day) - 8.8(day^2); R^2 = .79* (P=.05).

NO_3^--N levels were also lower after seven days possibly as a result of plant uptake.

Denitrification losses from urea, therefore, may be comparable to that of the NO_3^- fertilizers providing nitrification is first allowed to occur prior to the onset of soil conditions which favor denitrification. These losses would vary depending on the number of days of nitrification, rate of plant uptake, soil temperature and degree of soil saturation.

Denitrification in turf soils was influenced by soil factors such as texture, moisture content, temperature and fertilizer N addition. In particular, high soil temperatures (30 to 35 ^{o}C) and saturated soil conditions resulted in a high loss of applied N (40 to 85%) with larger losses associated with the finer textured soil. Low denitrification losses (less than 6% of applied N) occurred under a range of soil temperatures (22 to 30 ^{o}C) in association with unsaturated soil conditions (75% saturated) and saturated soil conditions in association with a moderate (22 ^{o}C) soil temperature.

C H A P T E R V

CONCLUSION

An extensive amount of research has been conducted over the past ten years in order to quantify and control denitrification losses from agricultural soils. Factors known to contribute to increased N-losses as a result of the denitrification process include additions of N fertilizers (particularly NO_3^--N sources), frequent and extensive irrigation and rainfall events, elevated soil temperatures, high levels of soil organic matter and increased levels of plant root and soil microbial respiration. The presence of large denitrifying populations may also contribute to a high potential for N-loss due to denitrification.

Intensively managed turf areas require large N fertilizer inputs and frequent, and often extensive, irrigations. Furthermore, recreational turfgrass areas typically have highly prolific and extensive root systems which contribute to increased levels of soil organic matter. A low turf mowing height may also result in increased soil temperatures. Denitrification N losses have, therefore, been assumed to occur from turf soils but have never been measured directly.

The results presented in this study represent the

first effort to directly measure denitrification losses from N fertilizers applied to turf. Preliminary work has shown that relatively large populations of denitrifying microorganisms exist in turfgrass soils indicating a high potential for denitrification. Denitrification losses from these turf soils were low, as in other cropped soils, until soil moisture levels approached saturation. Saturated soil conditions in combination with elevated soil temperatures resulted in very large denitrification losses from the N fertilizer applied. These conditions, in general, do not persist for long periods of time in soils but this and other research has shown that large denitrification losses may occur quickly and in relatively short time periods, e.g. one to two days.

Denitrification can never be completely eliminated as a form of N fertilizer fate but the coupling of results from laboratory and field studies will lead to an understanding of this process in turf soils and contribute to its control through the use of improved cultural practices. These practices would include proper rate and timing of N fertilizer application and irrigation, the use of proper N fertilizer materials and the maintenance of a well-aerated soil environment.

APPENDIX A

Acrylic Turf Chamber Construction

Nine acrylic chambers, with internal dimensions of 30.5 x 30.5 x 15.2 cm (L x W x D) and wall thicknesses of 1.27 cm were constructed (Figure 13). Seams between chamber walls and bottoms were glued with a two-part epoxy cement and then further secured with counter-sunk sheet metal screws (2.54 x 0.32 cm, L x D). The inside of each seam was made gas-tight with a silicon rubber sealant. Removable chamber lids were made of the same acrylic used for the chambers. A 2 cm diameter hole was drilled into the center of each lid and a rubber septum was sealed within the hole with silicon sealant.

Five copper tubes (28 x 0.4 cm, L x I.D.) were spaced every 5 cm apart in the bottom of each chamber beginning and ending 5 cm from the sidewalls. Prior to installation into a chamber, each copper tube was closed on one end by soldering and ten perforations (1.0 mm diameter) were drilled along the length of each tube. The perforations were spaced 2.54 cm apart and the end perforations were at least 5 cm from the chamber walls. Small plastic T-fittings were attached to the open ends of each pipe and used to connect them in parallel. Sidearms of the

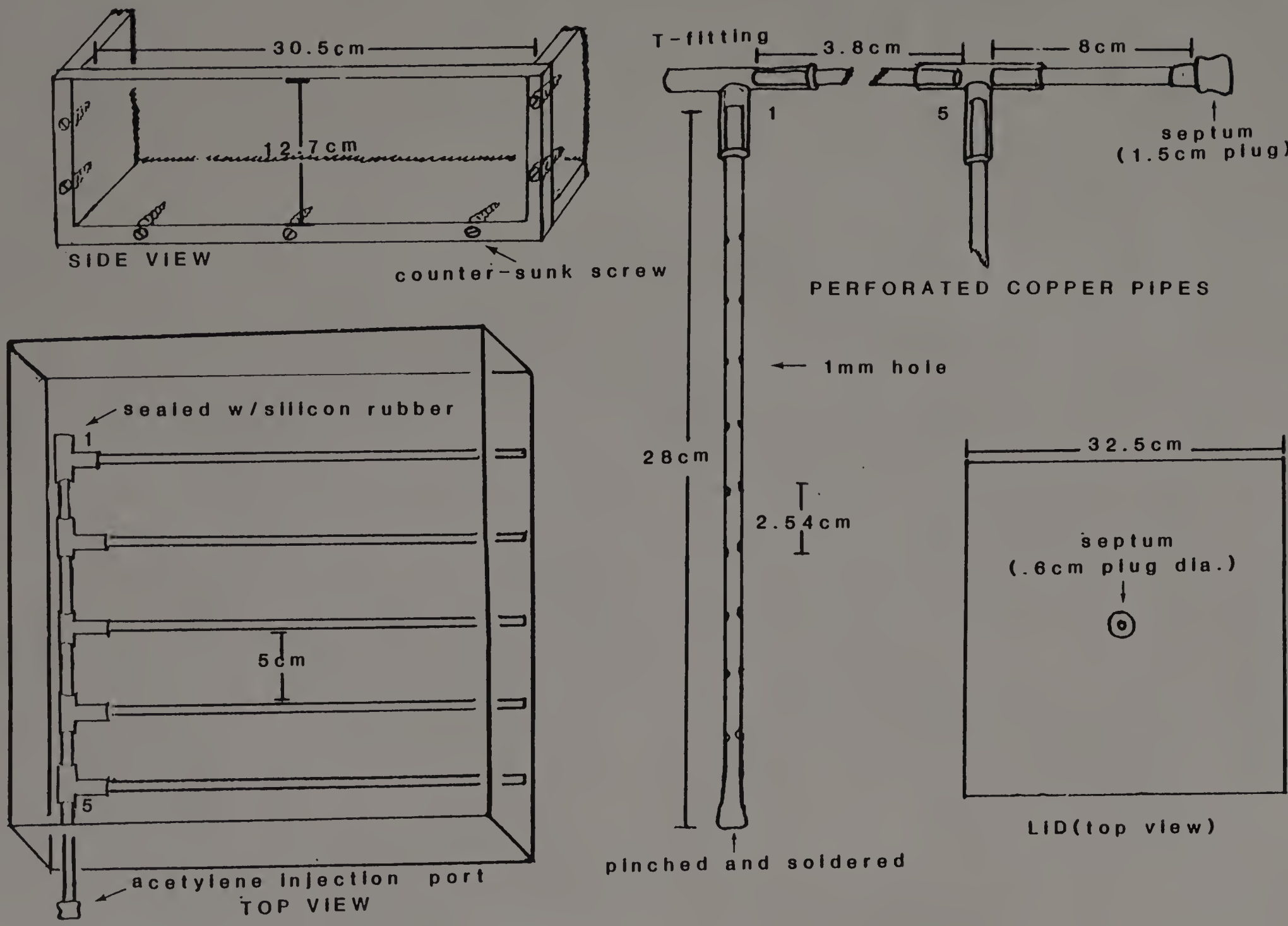

Figure 13. Acrylic turfgrass chamber.

T-fittings were connected with 4 cm lengths of copper tubing except for the outside sidearms of the first and last copper pipes. One outside sidearm was connected to an 8 cm length of copper pipe which extended through a hole in the chamber wall and was sealed with a rubber septum. This septum permitted C_2H_2 to be injected into the piping system. The other outside sidearm was sealed with silicon rubber sealant.

APPENDIX B

Chamber Volume and Leakage

All nine acrylic chambers were tested for leakage upon completion of construction. Acrylic lids were sealed onto the chambers with white lithium grease (Lubrimate Products, Omaha, Nebraska 68110) after which 100 ml of C_2H_2 were injected by syringe into each chamber through the septum in each lid. The syringe was pumped five times to thoroughly mix the C_2H_2 with the chamber air. A one ml gas sample was removed from each chamber after five minutes and initial (0 hour) C_2H_2 concentrations (ppm) were determined immediately by gas chromatography (Appendix E). Acetylene levels within each chamber were tested again after twelve hours. Percent chamber leakage was determined as [final acetylene concentration after 12 hours (ppm)/initial acetylene concentration (ppm)] x 100.

Leakage was unacceptable if C_2H_2 levels decreased by more than 5% during a twelve hour period. Chambers found to leak were sealed by recaulking all seams with silicon rubber sealant. This procedure was repeated until all chambers were determined to be air-tight (less than 5% leakage/12 hours). Chamber volume was also determined from this test and was equal to the [concentration (ppm) of pure

acetylene/ initial (0 hour) concentration (ppm) acetylene x 100 ml].

Final chamber volumes and % air leakage are shown in Table 13.

Table 13. Acrylic turf chamber volumes and % air leakage during a twelve hour period as determined by the change in C_2H_2 levels within the sealed chambers.

Chamber No.	C_2H_2 Concentration (ppm)		% Leakage	Chamber Volume (ml)
	0 hours*	12 hours		
1	8200	8009	2.33	12195
2	8453	8158	3.45	11830
3	8622	8320	3.50	11598
4	8376	7986	4.66	11939
5	8475	8195	3.30	11799
6	8445	8120	3.85	11841
7	8398	8162	2.81	11908
8	8259	8200	0.71	12108
9	8297	7967	3.98	12058

* Concentration immediately following C_2H_2 injection.

APPENDIX C

Installation of Sod into the Acrylic Chambers

Each acrylic chamber was placed upside down onto the Kentucky bluegrass turf and pressed firmly into the sod to form an outline of the chamber. The turf was then cut along this outline with an edging knife. The sod was cut to a soil depth of approximately 12.7 cm with a sod knife and removed from the ground. The sod sample was placed turf side down on plyboard and the soil surface was leveled with a sharpened spatula. The chamber was then placed over the sod sample to form the precise L x W dimensions that the sod was to be cut to. After the sod was cut to these dimensions, a wooden frame was placed over the sod which was then trimmed to a 7.6 cm depth. If done carefully, each sod sample could be cut uniformly in size and weight (approximately 7700 g ± 500 g, fresh weight).

One kilogram of fresh soil, previously trimmed from the bottoms of the sod samples, was uniformly distributed and pressed firmly into each chamber bottom. This soil was used to cover the perforated copper tubing used for C_2H_2 injection and allowed the sod sample to rest on a level surface. The sod was then carefully installed into each chamber and tamped firmly into place.

Small sod samples (12.7 x 12.7 x 12.7 cm, L x W x D) were also collected at the time of sod installation and were used to determine initial soil moisture content of sod samples by gravimetric means.

APPENDIX D

Determination of Air Volume in an Acrylic Chamber Containing Sod

The volume of air around a sod sample contained in an acrylic chamber was determined by rapidly pouring water into each chamber until an inverted miniscus was formed at the top of the each chamber wall. The miniscus for each chamber could be maintained for several minutes. Air volume (ml) was determined to be equal to the volume of water used to fill each chamber. Chamber headspace volumes were used to calculate total nitrous oxide-N loss from each sod sample.

An initial attempt was made to calculate air volume by injecting 50 ml of C_2H_2 (99% pure) into the headspaces of sealed chambers containing sod and then measuring the final C_2H_2 concentration (ppm) once an equilibrium was reached. Changes in C_2H_2 levels occured rapidly and equilibrium was not reached even after 300 minutes (Figure 14). This indicated that C_2H_2 will diffuse rapidly into sod even when injected into the chamber's headspace. It is interesting to note, however, that the C_2H_2 concentration obtained two minutes following injection approximated that of the volume obtained by the water volume technique

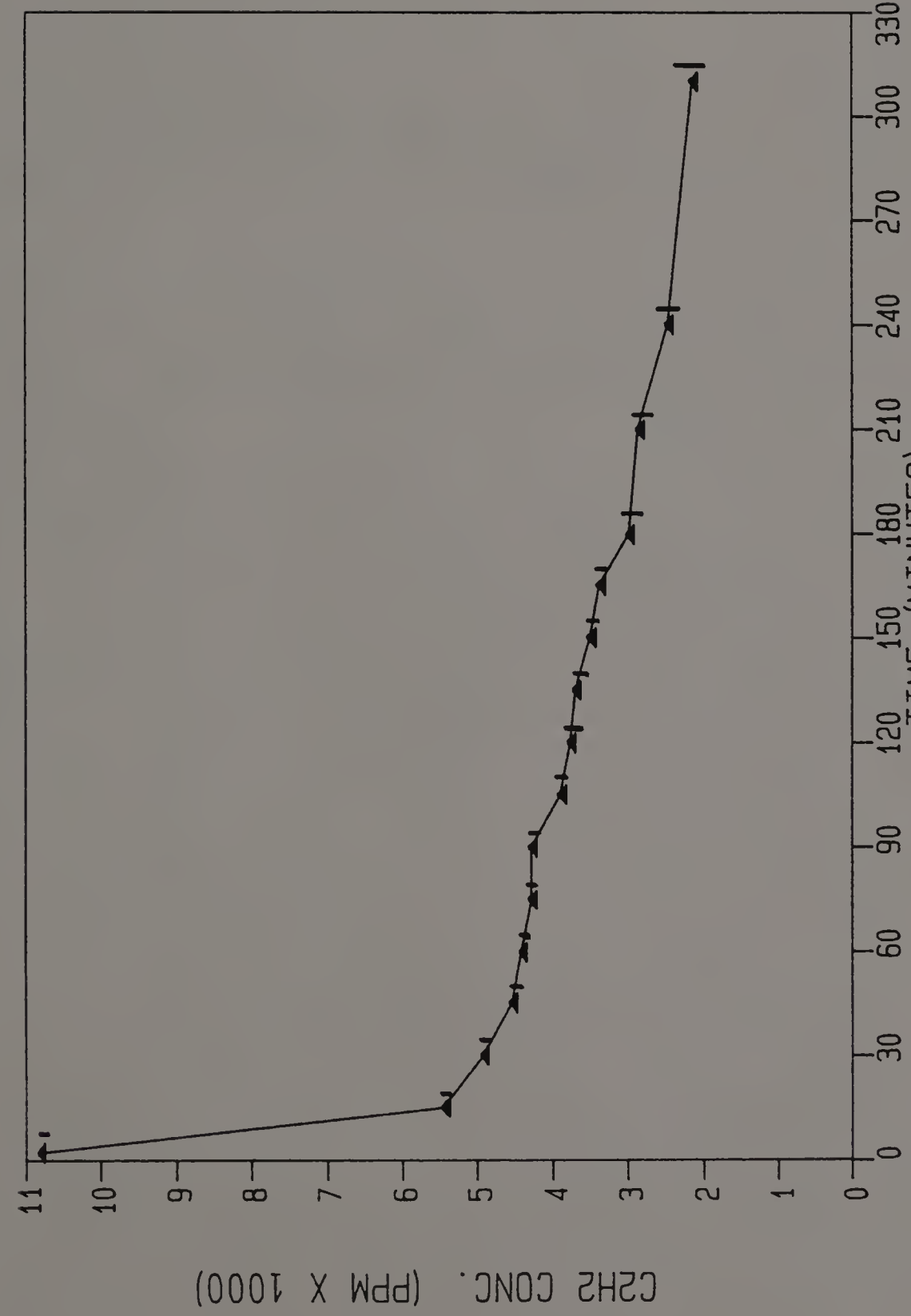

Figure 14. C_2H_2 levels following the injection of 50 ml of C_2H_2 into the headspace of acrylic turf chambers containing Kentucky bluegrass sod. Vertical lines represent standard error.

mentioned above. Using water, the mean air volume of six chambers was 4,998 ml (standard deviation ± 403.9) between six chambers while the C_2H_2 technique yielded a mean air volume of 4,823 ml (standard deviation ± 446.0) after 2 minutes. Changes in C_2H_2 concentrations occurred too rapidly and therefore could not be used to accurately determine air volumes in the sod chambers.

APPENDIX E

Determination of Acetylene

Acetylene levels were determined using a Shimadzu GC-Mini 2 gas chromatograph equipped with a flame ionization detector. Acetylene was separated grom other gases using an activated alumina column (80 mesh, 46 cm x 6.4 mm). Operational column temperature was 110 ^{o}C and injector port temperature was 145 ^{o}C. Nitrogen carrier gas was used at a column pressure of 1 kg/cm^{2}. Acetylene elution time was 1.58 minutes. The gas chromatograph's integrator (Shimadzu Chromatopac C-E1B) was calibrated with a 10,000 ppm C_2H_2 standard made with atomic absorption grade C_2H_2 (99% pure). Balance gas was N_2. Peak height, peak area and C_2H_2 concentration was determined by the integrator's computer.

APPENDIX F

The Diffusion of Acetylene Through the Sod Soil Profile

The diffusion of C_2H_2 through the sod was monitored in three chambers. A perforated copper tube was inserted vertically 18 cm into the sod soil through an opening in the chamber wall at a soil depth of 4 cm. Soil atmosphere samples were removed with a syringe through a rubber septum attached to the tube end on the outside of the chamber wall.

The sod used in this preliminary test was from the silt soil site and had a soil moisture content of 76% of saturation. Soil temperature was approximately 22 ^{o}C. One hundred ml of C_2H_2 (99% pure) was injected into each chamber via the C_2H_2 injection port (Figure 13). Headspace and soil gas samples were collected over a four hour period and analyzed for C_2H_2 concentration using gas chromatography (Appendix E).

The concentration of C_2H_2 in the soil atmosphere reached 14,110 ppm (1.41% v/v) in less than one hour following C_2H_2 introduction (Figure 15). This C_2H_2 level was approximately twice as high as the headspace level of C_2H_2 indicating that C_2H_2 was diffusing through the soil

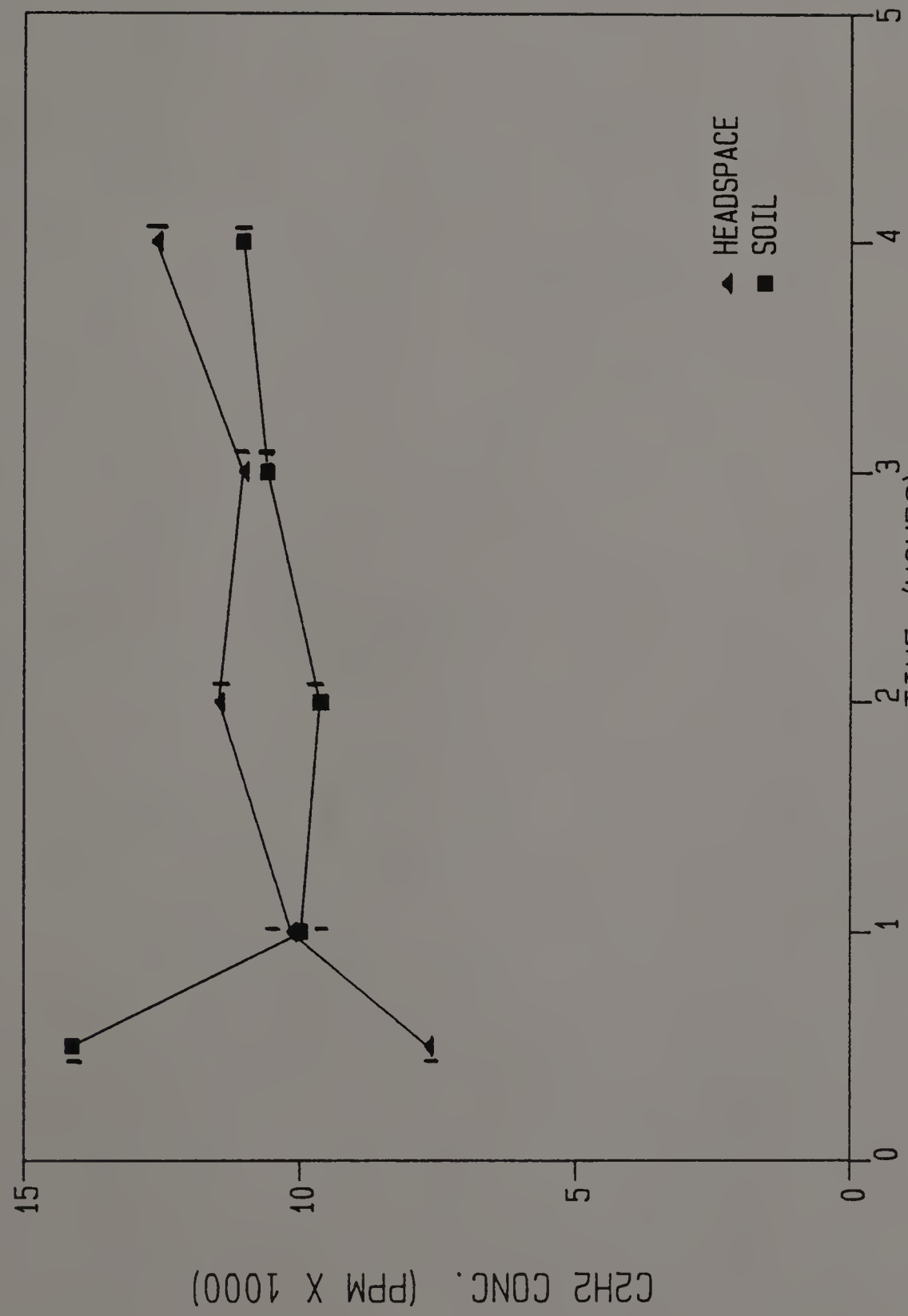

Figure 15. C_2H_2 diffusion through Kentucky bluegrass sod on a silt soil (22°C) and into the acrylic chamber headspace following injection of 100 ml C_2H_2 into the C_2H_2 injection ports (figure 13). Vertical lines represent standard error.

rather than along the chamber walls and into the headspace. A general equilibrium was reached between the soil and headspace for C_2H_2 concentrations after about one hour.

APPENDIX G

Determination of Nitrous Oxide-N

Nitrous oxide-N levels were determined using a Varian Series 2700 Moduline Gas Chromatograph (Palo Alto, CA 94303) equipped with an electron capture detector (Ti^3H). Nitrous oxide was separated from other gases using a Porapak Q column (80/100 mesh, 2 m x 3.2 mm). The column was preconditioned at 220 ^oC for 24 hours. Operational column temperature was 75 ^oC, injector port temperature was 195 ^oC and detector temperature was 220 ^oC. Nitrogen carrier gas was used at a flow rate of 14 ml/minute. Air (O_2, Ar, H_2), CO_2, N_2O and H_2O were separated into peaks evolved at 0.58, 1.18, 1.52 and 3.75 minutes, respectively. Time of elution was changed by carrier gas flow rate but sequence of elution was not affected. Detector response was linearly related to ng N_2O-N when N_2O levels ranged from 6.4 to 612.9 ng at a sensitivity setting (Attenuation x Amperage) of 64 x 10^{-10} and when levels ranged from 2.6 to 63.9 ng N_2O at a sensitivity of 16 x 10^{-10} (Figure 16). Standard curves were developed within these ranges from either a 107 ppm N_2O standard gas (Matheson Gas Products, Inc., Gloucester, MA 01930) or a 1000 ppm N_2O standard made with atomic absorption grade N_2O

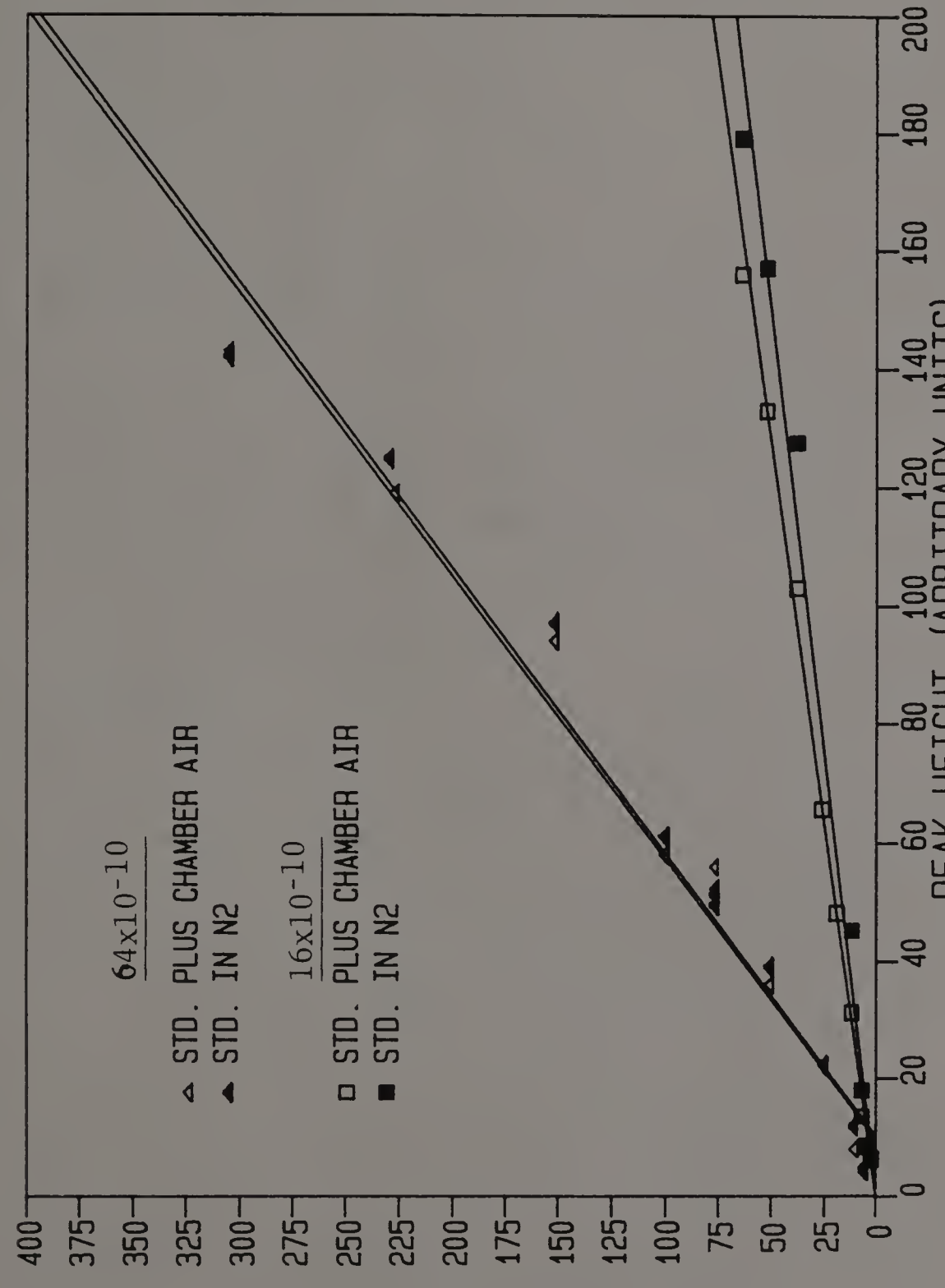

Figure 16. Response of $Ti^{3}H$ detector at two sensitivities to N_2O-N standards in either N_2 gas or headspace gas collected from acrylic turf chambers containing sod.

(99% pure). When attenuation was less than or equal to 16 x 10^{-10}, the detector responded similarly to N_2O in the presence or absence of air collected in the headspace above the sod for a 12 hour period. Headspace air had high concentrations of CO_2 and water vapor which influenced detector response to N_2O-N when detector sensitivity was greater than 16 x 10^{-10} (Figure 17).

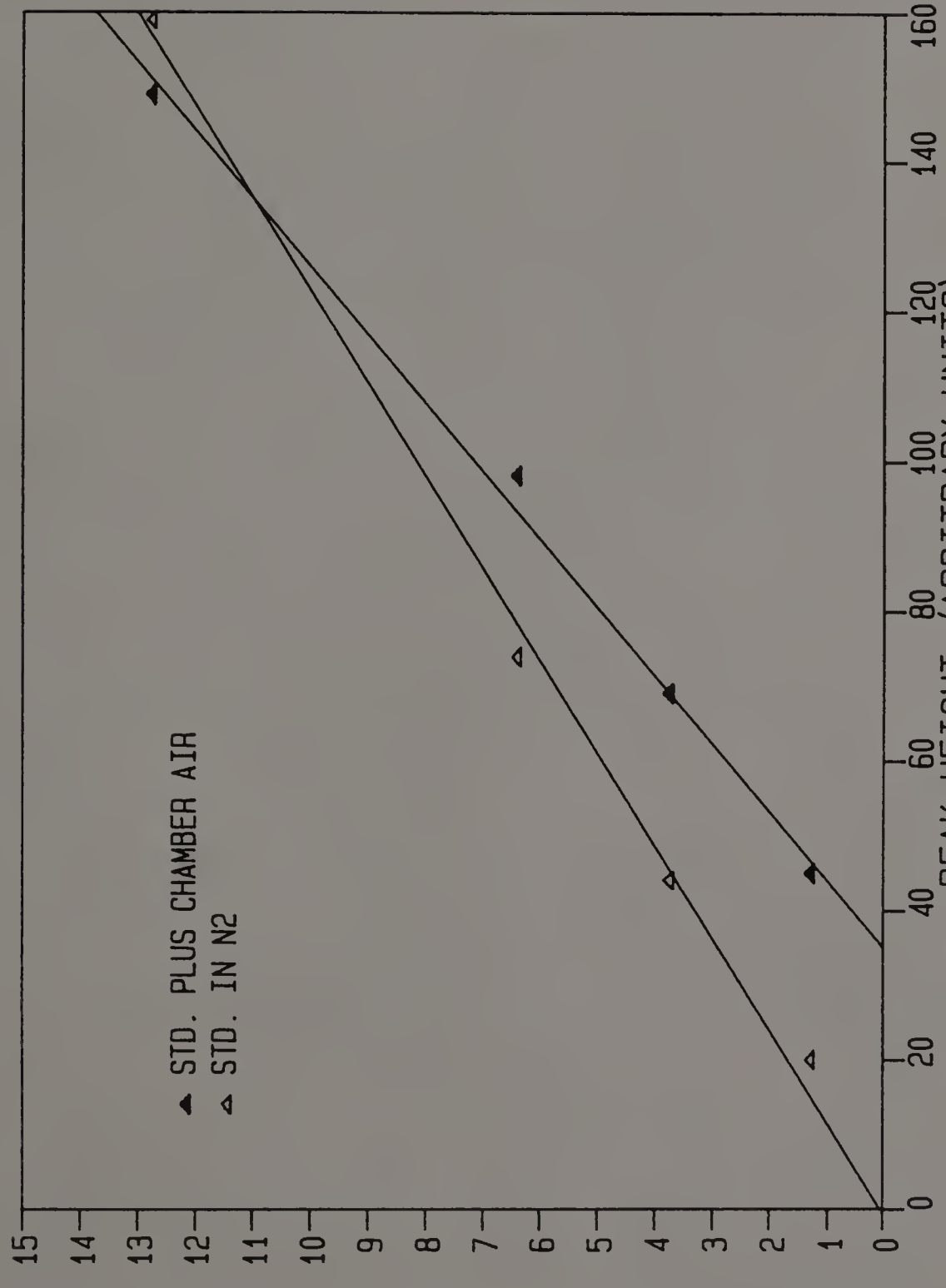

Figure 17. Response of $Ti^{3}H$ dectector (sensitivity = $4x10^{-10}$) to N_2O-N standards in either N_2 gas or headspace gas collected from acrylic turf chambers containing sod.

APPENDIX H

Headspace Gas Sampling During Denitrification Studies

The acrylic chambers were sealed following the application of water and/or fertilizer to the sod samples. The chamber lids were pressed lightly onto a bead of white lithium grease which had been applied to the lips of all chamber walls. Each chamber was inspected to insure that there were no gaps in the grease which would have allowed chamber air to escape. Following lid installation, C_2H_2 (100 ml) was injected into the chamber through the acetylene injection port (Figure 13).

The lids were kept on the chambers for twelve hour intervals. Three 1 ml headspace gas samples were removed from each chamber through the lid's septum prior to lid removal. Samples were collected with 1 ml syringes equipped with 13 mm needles (22 guage) and were deeply embedded imbedded into rubber stoppers to prevent gas leakage prior to analysis. Chamber lids were then removed for 0.5 hours to permit the chamber headspaces to ventilate. Air was also drawn through the turf thatch layer using a hand-held vacuum cleaner (Black and Decker Dust Buster, Model # 9330, Towson, MD). The chamber lids

were placed back onto the acrylic chambers and the procedure repeated again after 12 hours. These time intervals coincided with 12 hour light/dark periods.

Nitrous oxide-N content (ng N/1 ml) of the headspace gas samples was determined immediately after each collection time.

APPENDIX I

Total denitrification losses from Kentucky bluegrass sod on two soil types at various soil moisture levels following NO_3^--N addition (4886 mg N/m^2).

Soil Type	% Soil Saturation	mg N_2O-N/10day/m^2	% Applied N Lost
silt	65	1.2	<0.1
	70-82	4.0 ± 1.3*	<0.1
	83-85	23.3 ± 1.4	0.5
	86-90	43.2 ± 1.8	0.9
	91-95	51.8 ± 4.0	1.1
	96-100	147.1 ± 26.0	3.0
	106	245.6	5.0
silt loam	34	0.6	<0.1
	62	2.1	<0.1
	70-80	13.3 ± 9.1	0.3
	81-90	27.7 ± 9.3	0.6
	93	58.6	1.2
	101	107.0	2.2

* Mean ± standard deviation for specified soil moisture range.

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